Commission Regulation (EEC) No. 2676/90 determining Community methods for the analysis of wines

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Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Regulation (EEC) No 822/87 of 16 March 1987 on the common organization of the market in wine (1), as amended by Regulation (EEC) No 1325/90 (2), and in particular Article 74 thereof,

Whereas Article 74 (1) of Regulation (EEC) No 822/87 prescribes the adoption of methods of analysis for establishing the composition of the products indicated in Article 1 of that Regulation and of rules for checking whether these products have been subjected to treatments in violation of authorized oenological pratice;

Whereas, inasmuch as the Community has not yet laid down maximum levels for substances whose presence indicates that certain oenological practices have been used and has not yet adopted tables enabling analysis data to be compared, there is good reason to authorize Member States to determine such maximum levels;

Whereas Article 13 (1) of Regulation (EEC) No 822/87 provides for an analytical test, including at the least an assessment of the characteristics, as listed in the Annex to that Regulation, of the quality wine psr in question;

Whereas the verification of the particulars on documents concerning the products in question calls for the introduction of uniform methods of analysis to ensure that accurate and comparable information is obtained; whereas, consequently, these methods should be compulsory for all commercial transactions and all verification procedures; whereas, however, in view of the trade's limited facilities, a restricted number of usual procedures should be admitted enabling the requisite factors to be determined rapidly and with reasonable accuracy;

Whereas, as far as is possible, generally recognized methods such as those developed under the 1954 International Convention for the Unification of Methods of Analysis and Appraisal of Wines, which are published in the Recueil des méthodes internationales d'analyse des vins (Compendium of international methods for the analysis of wines) by the International Office of Vine and Wine, may usefully be retained;

Whereas the Community methods of analysis applicable to wine were laid down in Commission Regulation (EEC) No 1108/82 (3); whereas scientific progress has made it necessary to replace certain of the methods by some that are more suitable, to modify others, and to introduce new methods, particularly those approved since the aforesaid Regulation by the International Office of Vine and Wine; whereas, because of the profusion and complexity of these changes, all the analyses should be reassembled in a new Regulation, and Regulation (EEC) No 1108/82 should be repealed;

Whereas, in order to ensure the comparability of the results obtained by applying the analytical methods referred to in Article 74 of Regulation (EEC) No 822/87, steps should be taken to refer, in regard to the accuracy, repeatability and reproducibility of these results, to the definitions laid down by the International Office of Vine and Wine;

Whereas, in order to recognize the scientific advances on the one hand and the technical equipment of official laboratories on the other, and with the aim of increasing the efficiency and profitability of these laboratories, there is good reason to allow automated analytical methods to be applied under certain conditions; whereas it is important to specify that, where a dispute arises, the automated methods may not replace the reference methods and the usual methods;

Whereas the results of a density measurement using the automated method based on the principle of the frequency oscillator are, in respect of their accuracy, repeatability and reproducibility, at least as good as the results obtained by the methods listed in section 1 of the Annex to the present Regulation for measuring the density or specific gravity; whereas it is therefore indicated, by virtue of Article 74 (3) of Regulation (EEC) No 822/87, that this automated method shall be considered as equivalent to the said methods listed in the Annex to the present Regulation;

Whereas the procedure described in Chapter 25 under point 2.2.3.3.2 in the Annex hereto for analysing the total sulphur dioxide content of wines and grape musts of a presumed content of less than 50 mg/l results in better extraction of that substance compared to the methods described in Chapter 13 under point 13.4 of the Annex to Regulation (EEC) No 1108/82; whereas the result is higher total sulphur dioxide contents of the products analysed, which may exceed, in particular, in the case of certain grape juices, the maximum limit laid down; whereas, in order to avoid difficulties in the disposal of grape juice already prepared at the time of entry into force of this Regulation and until such time as the production processes are adapted to result in more complete de-sulphiting of grape musts with fermentation arrested by the addition of alcohol, the procedure described in the abovementioned Regulation should be allowed to be used during a transitional period;

Whereas the measures provided for in this Regulation are in accordance with the opinion of the Management Committee for Wine,

HAS ADOPTED THIS REGULATION:

Article 1

1. The Community methods for the analysis of wine making it possible, in the context of commercial transactions and all control operations, to:

- establish the composition of the products listed in Article 1 of Regulation (EEC) No 822/87,

- check whether these products have been subjected to treatments in violation of authorized oenological practice,

are those set out in the Annex to this Regulation.

2. For substances for which reference methods and usual methods are prescribed, the results obtained by the use of the reference methods shall prevail.

Article 2

For the purposes of applying this Regulation:

(a) the repeatability shall be the value below which the absolute difference between the two single test results obtained on identical test material, under the same conditions (same operator, same apparatus, same laboratory and a short interval of time), may be expected to lie with a specified probability;

(b) the reproducibility shall be the value below which the absolute difference between two single test results obtained identical test material, under different conditions (different operators, different apparatus and/or different laboratories and/or different time), may be expected to lie with a specified probability.

The term 'single test result' shall be the value obtained when the standardized test method is applied fully and once to a single sample.

Unless otherwise stated, the probability shall be 95 %.

Article 3

1. Automated analytical methods shall be acceptable, under the responsibility of the Director of a laboratory on condition that the accuracy, repeatability and reproducibility of the results are at least equivalent to those of the results obtained by the analytical methods listed in the Annex.

Where a dispute arises, the methods listed in the Annex may not be replaced by automated methods.

2. The automated method for measuring density based on the principle of the frequency oscilliator shall be considered as equivalent to the methods listed in section 1 of the Annex to the present Regulation.

Article 4

Wherever mention is made of water for solution, dilution, or washing purposes, this shall mean distilled water or demineralized water of equivalent purity. All chemicals shall be of analytical reagent quality except where otherwise specified.

Article 5

Regulation (EEC) No 1108/82 is repealed.

However Article 1 (4) of that Regulation shall apply until 31 December 1990.

Article 6

This Regulation shall enter into force on the day of its publication in the Official Journal of the European Communities.
It shall apply with effect from 1 October 1990.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 17 September 1990.

For the CommissionRay MAC SHARRYMember of the Commission

(1)OJ No L 84, 27. 3. 1987, p. 1. (2)OJ No L 132, 23. 5. 1990, p. 19. (3)OJ No L 133, 14. 5. 1982, p. 1.

ANNEX
1. DENSITY AND SPECIFIC GRAVITY AT 20 °C 1. DEFINITIONS

The density is the mass per unit volume of wine or must at 20 °C. It is expressed in grams per millilitre and denoted by the symbol r 20 °C.

The specific gravity at 20 °C (or the 20 °C/20 °C relative density) is the ratio, expressed as a decimal number, of the density of a certain volume of the wine or must at 20 °C to the density of same volume water at the same temperature. It is denoted by the symbol d20 °C
20 °C
. 2. PRINCIPLE OF METHODS

The density and specific gravity at 20 °C are measured on a test sample:

- either by pigmentary: reference method,

- or by hydrometry or densimetry using a hydrostatic balance: usual methods.

Note:For very accurate measurement, the density must be corrected for the effect of sulphur dioxide by using the formula:

r20 °C = r220 °C 0,0006 × Swhere r20 °C = corrected densityr220 °C = observed densityS = total quantity of sulphur dioxide in grams per litre3. PRELIMINARY TREATMENT OF SAMPLE

If the wine or must contains appreciable quantities of carbon dioxide, remove most of it by stirring 250 ml of the wine in a 1-litre flask or by filtration under reduced pressure through 2 g of cotton wool placed in an extension tube.4. REFERENCE METHOD

4.1. Apparatus:Normal laboratory equipment, and in particular:

4.1.1. A Pyrex pycnometer (1), of approximately 100 ml capacity, with a removable ground-glass jointed thermometer calibrated in tenths of a degree from 10 to 30 °C (Figure 1). The thermometer must be standardized.

Figure 1 The pycnometer and its tare

The pycnometer has a side tube 25 mm in length and 1 mm (maximum) in internal diameter, ending in a conical ground joint. This side tube may be capped by a 'reservoir stopper' consisting of a conical ground-glass joint tube and terminating in a drawn-out section. This stopper serves as an expansion chamber.

The two ground joints of the apparatus should be prepared with great care.

4.1.2. A tare bottle, consisting of a vessel with the same outside volume (to within at least 1 ml) as the pycnometer and with a mass equal to the mass of the pycnometer filled with a liquid of specific gravity 1,01 (sodium chloride solution 2,0 % m/v).

A thermally insulated container, exactly fitting the body of the pycnometer.

4.1.3. A twin-pan balance with a range of at least 300 g and a sensitivity of 0,1 mg,ora single-pan balance with a range of at least 200 g and a sensitivity of 0,1 mg.4.2. Calibration of pycnometer

Calibration of the pycnometer involves a determination of the following quantities:- tare of the empty pycnometer,- volume of the pycnometer at 20 °C,- mass of the water-filled pycnometer at 20 °C.4.2.1. Method using a twin-pan balance

Place the tare bottle on the left-hand pan of the balance and the clean and dry pycnometer, fitted with its 'reservoir stopper', on the right-hand pan. Add weights to the pan holding the pycnometer and record the weight required to establish equilibrium: let it be p grams.

Carefully fill the pycnometer with distilled water at the ambient temperature and fit the thermometer; carefully wipe the pycnometer dry and place it in the thermally insulated container. Mix it by inverting the container until the temperature reading on the thermometer is constant. Accurately adjust the level to the upper rim of the side tube. Wipe the side tube dry and put the reservoir stopper on; read the temperature t°C carefully, possibly correcting it for the inaccuracy in the temperature scale. Weigh the water-filled pycnometer against the tare and record the weight p2 in grams required to establish equilibrium.

Calculation (2):

Taring of empty pycnometer:tare of empty pycnometer =p + m,where m = mass of air contained in the pycnometer,m = 0,0012 (p p2).

Volume at 20 °C:V20 °C = (p + m p2) · Ft,where Ft = a factor taken from Table I for the temperature t °C.V20 °C should be known to within ± 0,001 ml.

Mass of water at 20 °C:M20 °C = 0,998203 V20 °C,where 0,998203 is the density of water at 20 °C.
4.2.2. Method using a single-pan balance

Determine:- the mass of the clean and dry pycnometer: let this be P,- the mass of the pycnometer filled with water at t °C, following the procedure described in 4.2.1 above: let this be P1,- the mass of the tare: T0.

Calculation (3):

Taring of empty pycnometer:tare of empty pycnometer =P m,where m = mass of air contained in the pycnometer,m = 0,0012 (P1 P).

Volume at 20 °C:V20 °C = [P1 (P m)] × Ftwhere Ft= a factor taken from Table I for the temperature t °C.The volume at 20 °C should be known to within ± 0,001 ml.

Mass of water at 20 °C:M20 °C = 0,998203 V20 °C,where 0,998203 is the density of water at 20 °C.4.3. Method of measurement (3)

4.3.1. Method using twin-pan balance

Fill the pycnometer with the prepared test sample, following the procedure described in 4.2.1 above.

Let p" be the weight in grams required to establish equilibrium at t °C.

Mass of liquid contained in the pycnometer = p + m p".

Apparent density at t °C:

Calculate the density at 20 °C using one of the correction tables given later, in accordance with the nature of the liquid being measured: dry wine (Table II), natural or concentrated must (Table III), sweet wine (Table IV).

The 20 °C/20 °C specific gravity of the wine is calculated by dividing its density at 20 °C by 0,998203.4.3.2. Method using a single-pan balance (3)

Weigh the tare bottle and let its mass be T.Calculate dT = T1 T0.Mass of the empty pycnometer at the time of measurement = P m + dT.

Weigh the pycnometer filled with the prepared test sample, following the procedure described in 4.2.1 above. Let its mass att °C be P2.

Mass of liquid contained in pycnometer at t °C = P2 (P m + dT)

Apparent density at t °C:

Calculate the density at 20 °C of the liquid under test (dry wine, natural or concentrated must, or sweet wine) as indicated in 4.3.1 above.

The 20 °C/20 °C specific gravity is calculated by dividing the density at 20 °C by 0,998203.

4.3.3. The repeatability of the density measurementsfor dry and semi-sweet wines: r = 0,00010,

and for sweet wines: r = 0,00018.

4.3.4. The reproducibility of the density measurements:for dry and semi-sweet wines: R = 0,00037,and for sweet wines: R= 0,00045.5. USUAL METHODS

5.1. Hydrometry

5.1.1. Apparatus

5.1.1.1. Hydrometer

Hydrometers shall conform to ISO Standards as regards their dimensions and graduation.

They shall have a cylindrical bulb, and a stem of circular cross-section with a minimum diameter of 3 mm. For dry wines, they shall be graduated from 0,983 to 1,003 with graduation marks every 0,0010 and 0,0002. Each mark at 0,0010 shall be separated from the next corresponding mark by at least 5 mm. For measuring the density of de-alcoholized wines, sweet wines and musts, a set of five hydrometers graduated from 1,000 to 1,030, 1,030 to 1,060, 1,060 to 1,090, 1,090 to 1,120 and 1,120 to 1,150 shall be used. These hydrometers shall be graduated in densities at 20 °C with marks at least every 0,0010 and 0,0005, each mark at 0,0010 being separated by at least 3 mm from the next corresponding mark.

These hydrometers shall be graduated so as to be read 'at the top of the meniscus'. An indication of the graduation in density or specific gravity at 20 °C and of the reading at the top of the meniscus shall be carried either on the graduated scale or on a slip of paper enclosed in the bulb. These hydrometers shall be calibrated by a government department.

5.1.1.2. A calibrated thermometer graduated in minimum intervals of 0,5 °C.

5.1.1.3. A measuring cylinder of 36 mm internal diameter and 320 mm height, held vertical by supporting levelling screws.5.1.2. Procedure

5.1.2.1. Method of measurement

Pour 250 ml of the test sample, prepared as in 5.1.1.3 above, into the measuring cylinder and introduce the thermometer and hydrometer into it. Read the temperature one minute after mixing the sample well to produce a uniform temperature. Remove the thermometer and read the apparent density at t °C from the hydrometer after a further minute.

The apparent density at t °C is then corrected to 20 °C by using the tables applicable to dry wines (Table V), musts (Table VI) or wines containing sugars (Table VII).

The 20 °C/20 °C specific gravity is obtained by dividing the density at 20 °C by 0,998203.5.2. Densimetry using a hydrostatic balance

5.2.1. Apparatus

5.2.1.1. Hydrostatic balance with a maximum capacity of at least 100 g and a sensitivity of 0,1 mg.

Identical Pyrex glass floats with a volume of at least 20 ml are fixed under both pans by suspending them with a thread of diameter not exceeding 0,1 mm.

The float suspended under the right-hand pan must be capable of being introduced into a measuring cylinder bearing a mark indicating the level. The measuring cylinder must have an internal diameter at least 6 mm larger than that of the float. The float must be capable of being contained completely within the volume of the measuring cylinder located below the mark; the surface of the liquid to be measured must be penetrated only by the supporting thread. The temperature of the liquid in the measuring cylinder is measured by a thermometer graduated in steps of 0,2 °C.

5.2.1.2. A single-pan hydrostatic balance may also be used.5.2.2. Procedure

5.2.2.1. Standardization of a hydrostatic balance

With the two floats in air, establish balance by placing weights in the right-hand pan. Record the mass p of these added weights.

Fill the measuring cylinder with pure water up to the mark and read the temperature t °C after shaking and allowing to stand for two or three minutes.Re-establish balance by placing weights in the right-hand pan. Let the mass of these bep2.

The volume of the float at 20 °C is given by:V20 °C = (p2 p) (F + 0,0012)where F is the factor given in Table I for the temperature t °C.
p and V20 °C are the characteristics of the float.5.2.2.2. Method of measurement

The right-hand float is immersed in the measuring cylinder filled with wine (or must) up to the mark. The temperature t °C of the wine (or must) is read and the massp" needed to re-establish balance is recorded.

The apparent density rt is given byThis density is corrected to 20 °C by using one of the Tables II, III or IV if the float is of Pyrex glass.
6. EXAMPLE OF THE CALCULATION OF THE DENSITY AT 20 °C AND THE 20 °C/20 °C SPECIFIC GRAVITY (REFERENCE METHOD)

6.1. Pycnometry using a twin-pan balance

6.1.1. Standardization of the pycnometer:

1. Weighing of clean dry pycnometer:Tare = pycnometer + p
p = 104,9454 g

2. Weighing of pycnometer filled with water at t °C:Tare = pycnometer + water + p2
p2 = 1,2396 g att = 20,5 °C

3. Calculation of mass of air contained in the pycnometer:m = 0,0012 (p p2)
m = 0,0012 (104,9454 1,2396)
m = 0,1244 g

4. Characteristic values to be retained:Tare of empty pycnometer, p + m:
p + m = 104,9454 + 0,1244
p + m = 105,0698 gVolume at 20 °C = F (p + m p2)t °CF20,5 °C = 1,001900
V20 °C = (105,0698 1,2396) × 1,001900
V20 °C = 104,0275 mlMass of water at 20 °C =
M20 °C = V20 °C · 0,998203
M20 °C = 103,8405 g6.1.2. Determination of the density and specific gravity at 20 °C/20 °C of a dry wine

r" = 1,2622 at 17,80 °Cr17,80 °C = 0,99788 g/ml

Table II enables r20 °C to be calculated from rt °C using the relationship:

For t = 17,80 °C and for an alcoholic strength of 11 % vol. c= 0,54.6.2. Pycnometry using a single-pan balance

6.2.1. Standardization of the pycnometer:

1. Weight of clean dry pycnometer:P = 67,7913 g

2. Weight of pycnometer filled with water at t °C:P1 = 169,2715 at 21,65 °C

3. Mass of air contained in the pycnometer:m = 0,0012 (P1 P)
m = 0,0012 × 101,4802
m = 0,1218 g

4. Characteristic values to be retained:Tare of empty pycnometer, P m:
P m= 67,7913 0,1218
P m = 67,6695 gVolume at 20 °C = [P1 (P m)] Ft °C
F21,65 °C = 1,002140
V20 °C = 1,002140 (169,2715 67,6695)
V20 °C = 101,8194 mlMass of water at 20 °C:
M20 °C = V20 °C × 0,998203
M20 °C = 101,6364 gMass of tare bottle, To:
To = 171,9160 g6.2.2. Determination of the density and specific gravity at 20 °C of a dry wine

T1 = 171,9178 g
dT = 171,9178 171,9160 = 0,0018 g
P m + dT = 67,6695 + 0,0018 = 67,6713 g
P2 = 169,2799 at 18 °Cr18 °C = 0,99793 g/ml

Table II enables r20 °C to be calculated from rt °C using the relationship:

For t= 18 °C and an alcoholic strength of 11 % vol. c = 0,49.

TABLE I F factors by which the mass of water contained in the Pyrex pycnometer at t °C has to be multiplied in order to calculate the pycnometer volume at 20 °C

TABLE II Temperature corrections c to the density of alcohol-free dry wines measured with a Pyrex glass pycnometer at t °C to relate the result to 20 °C

TABLE III Temperature corrections cto the density of natural musts and of concentrated musts measured with a Pyrex glass pycnometer at t °C to relate the result to 20 °C

TABLE IV Temperature corrections c to the density of wines of 13 % vol and above containing residual sugar measured with a Pyrex glass pycnometer at t °C to relate the result to 20 °C

TABLE V Temperature corrections c to the density of dry wines and alcohol-free dry wines measured with an ordinary glass pycnometer or hydrometer at t °C to relate the result to 20 °C

TABLE VI Temperature corrections cto the density of natural musts and of concentrated musts measured with a pycnometer or hydrometer of ordinary glass att °C to relate the result to 20 °C

TABLE VII Temperature corrections cto the density of wines of 13 % vol and above containing residual sugar measured with a hydrometer or pycnometer of ordinary glass at t °C to relate the result to 20 °C

2. EVALUATION BY REFRACTOMETRY OF THE SUGAR CONCENTRATION IN GRAPE MUSTS, CONCENTRATED GRAPE MUSTS AND RECTIFIED CONCENTRATED GRAPE MUSTS 1. PRINCIPLE OF THE METHOD

The refractive index at 20 °C, expressed either as an absolute value or as a percentage by mass of sucrose, is given in the appropriate table to provide a means of obtaining the sugar concentration in grams per litre and in grams per kilogram for grape musts, concentrated grape musts and rectified concentrated grape musts.2. APPARATUS

2.1. Abbé refractometer

The refractometer used must be fitted with a scale giving:- either percentage by mass of sucrose to 0,1 %,- or refractive indices to four decimal places.

The refractometer must be equipped with a thermometer having a scale extending at least from +15 °C to +25 °C and with an arrangement for circulating water enabling measurements to be made at a temperature of 20 ± 5 °C.

The operating instructions for this instrument must be strictly adhered to, particularly with regard to calibration and the light source.3. PREPARATION OF THE SAMPLE

3.1. Must and concentrated must

Pass the must, if necessary, through a dry gauze folded into four and, after discarding the first drops of the filtrate, carry out the determination on the filtered product.3.2. Rectified concentrated must

Depending on the concentration, use either the rectified concentrated must itself or a solution obtained by making up 200 g of rectified concentrated must to 500 g with water, all weighings being carried out accurately.4. PROCEDURE

Bring the sample to a temperature close to 20 °C. Place a small test sample on the lower prism of the refractometer, taking care (because the prisms are pressed firmly against each other) that this test sample covers the glass surface uniformly. Carry out the measurement in accordance with the operating instructions of the instrument used.

Read off the percentage by mass of sucrose to within 0,1 % or read the refractive index to four decimal places.

Carry out at least two determinations on the same prepared sample.

Note the temperature t °C.5. CALCULATION

5.1.Temperature correction

5.1.1. Instruments graduated in percentage by mass of sucrose: use Table I to obtain the temperature correction.

5.1.2. Instruments graduated in refractive index: find the index measured at t °C in Table II to obtain (column 1) the corresponding value of the percentage by mass of sucrose at t °C. This value is corrected for temperature and expressed as a concentration at 20 °C by means of Table I.5.2. Sugar concentration in must and concentrated must

Find the percentage by mass of sucrose at 20 °C in Table II and read from the same row the sugar concentration in grams per litre and grams per kilogram. The sugar concentration is expressed in terms of invert sugar to one decimal place.5.3. Sugar concentration in rectified concentrated must

Find the percentage by mass of sucrose at 20 °C in Table III and read from the same row the sugar concentration in grams per litre and grams per kilogram. The sugar concentration is expressed in terms of invert sugar to one decimal place.

If the measurement was made on diluted rectified concentrated must, multiply the result by the dilution factor.5.4. Refractive index of must, concentrated must and rectified concentrated must

Find the percentage by mass of sucrose at 20 °C in Table II and read from the same row the refractive index at 20 °C. This index is expressed to four decimal places.
TABLE I Correction to be made when the percentage by mass of sucrose has been determined at a temperature different from 20 °C

TABLE II Table giving the sugar concentration (4) in must and concentrated must in grams per litre and grams per kilogram, determined by means of a refractometer graduated either in percentage by mass of sucrose at 20 °C or in refractive index at 20 °C. The density at 20 °C is also given

TABLE III Table giving the sugar (5) concentration in rectified concentrated must in grams per litre and grams per kilogram, determined by means of a refractometer graduated either in percentage by mass of sucrose at 20 °C or in refractive index at 20 °C. The density at 20 °C is also given

3. ALCOHOLIC STRENGTH BY VOLUME 1. DEFINITION

The alcoholic strength by volume is the number of litres of ethanol contained in 100 litres of wine, both volumes being measured at a temperature of 20 °C. It is expressed by the symbol '% vol'.

Note:Homologues of ethanol, together with the ethanol and ethanol homologues in ethyl esters, are included in the alcoholic strength since they occur in the distillate.2. PRINCIPLE OF METHODS

2.1. Distillation of wine made alkaline by a suspension of calcium hydroxide. Measurement of the alcoholic strength of the distillate.

2.2. Reference method: measurement of the density of the distillate with a pycnometer.2.3. Usual methods:

2.3.1. Measurement of the alcoholic strength of the distillate with a hydrometer.

2.3.2. Measurement of the alcoholic strength of the distillate by densimetry using a hydrostatic balance.

2.3.3. Measurement of the alcoholic strength of the distillate by refractometry.

Note:To obtain the alcoholic strength from the density of the distillate, use Tables I, II and III in Appendix II to this section of the Annex. These have been calculated from the International Tables of Alcoholic Strength published in 1972 by the International Legal Metrology Organization in its Recommendation 22 and adopted by the OIV (General Assembly, 1974).Table I gives the general formula relating the alcoholic strength by volume and the density of alcohol-water mixtures as a function of temperature.3. METHOD OF OBTAINING DISTILLATE

3.1. Apparatus

3.1.1. Distillation apparatus, consisting of:- a round-bottomed 1-litre flask with ground-glass joints,- a rectifying column about 20 cm in height or any apparatus to prevent splashing,- a source of heat; any pyrolysis of extracted matter must be prevented by a suitable arrangement,- a condenser terminated by a drawn-out tube taking the distillate to the bottom of a graduated receiving flask containing several ml of water.

3.1.2. Steam-distillation apparatus consisting of:1. a steam-generator; 2. a steam pipe; 3. a rectifying column; 4. a condenser.

Any type of distillation or steam-distillation apparatus may be used provided that it satisfies the following test:Distil an ethanol-water mixture with an alcoholic strength of 10 % vol five times in succession. The distillate should have an alcoholic strength of at least 9,9 % vol after the fifth

distillation, i.e. the loss of alcohol during each distillation should not be more than 0,02 % vol.3.2. Reagents

3.2.1. A 2 M suspension of calcium hydroxide, obtained by carefully pouring 1 litre of water at 60 to 70 °C on to 120 g of quicklime (CaO).3.3. Preparation of sample

Remove the bulk of any carbon dioxide from young and sparkling wines by stirring 250 to 300 ml of the wine in a 500-ml flask.3.4. Procedure

Measure out 200 ml of the wine using a graduated flask.

Record the temperature of the wine.

Transfer the wine to the distillation flask and introduce the steam-pipe of the steam-distillation apparatus. Rinse the graduated flask four times with successive 5-ml washings of water added to the flask or the steam-pipe. Add 10 ml of (3.2.1) calcium hydroxide and several pieces of inert porous material (pumice, etc).

Collect the distillate in the 200-ml graduated flask used to measure the wine.

Collect a volume of about three-quarters of the initial volume if distillation is used and a volume of 198 to 199 ml of distillate if steam distillation is used. Make up to 200 ml with distilled water, keeping the distillate at a temperature within 2 °C of the initial temperatures.

Mix with great care, using a circular motion.

Note:In the case of wines containing particularly large concentrations of ammonium ions, the distillate may be redistilled under the conditions described above, but replacing the suspension of calcium hydroxide with 1 M sulphuric acid diluted to 10 parts in 100 (vol/vol).4. REFERENCE METHOD

Measurement of the alcoholic strength of the distillate using a pycnometer.4.1. Apparatus

4.1.1. Use the standardized pycnometer as described in the chapter 'Density and specific gravity' (Annex, chapter 1).4.2. Procedure

Measure the apparent density of the distillate (3.4) at t °C as described in the chapter 'Density and specific gravity' (Annex, chapter 1, sections 4.3.1 and 4.3.2). Let this density be rt.4.3. Expression of results

4.3.1. Method of calculation

Find the alcoholic strength at 20 °C using Table I. In the horizontal line of this table corresponding to the temperature T (expressed as a whole number) immediately below t °C, find the smallest density greater than rt. Use the tabular difference just below this density to calculate the density r at this temperature T.

On the line of the temperatureT, find the density r immediately above r2 and calculate the difference between the densities rand r2. Divide this difference by the tabular difference just to the right of the density r2.The quotient gives the decimal part of the alcoholic strength, while the

whole number part of this strength is shown at the head of the column in which the density r2 is located.

An example of the calculation of an alcoholic strength is given in Appendix I to this chapter of the Annex.

Note:This temperature correction has been incorporated in a computer program and might possibly be carried out automatically.4.3.2. Repeatability, r: r = 0,10 % vol.4.3.3. Reproducibility, R: R = 0,19 % vol.5. USUAL METHODS

5.1. Hydrometry

5.1.1. Apparatus

5.1.1.1. Alcoholmeter.

The alcoholmeter must conform to the specifications for class I or class II equipment defined in International Recommendation No 44, Alcoholmeters and Alcohol Hydrometers, of the ILMO (International Legal Metrology Organization).

5.1.1.2. Thermometer graduated in degrees and in 0,1 °C from 0 to 40 °C certified to within 1/20th degree.

5.1.1.3. Measuring cylinder of diameter 36 mm and height 320 mm held vertically by supporting levelling screws.5.1.2.Procedure

Pour the distillate (3.4) into the measuring cylinder. Ensure that the cylinder is kept vertical. Insert the thermometer and alcoholmeter. Read the temperature on the thermometer one minute after stirring to equilibrate the temperature of the measuring cylinder, the thermometer, the alcoholmeter and the distillate. Remove the thermometer and read the apparent alcoholic strength after one minute. Take at least three readings using a magnifying glass. Correct the apparent strength measured at t °C for the effect of temperature using Table II.

The temperature of the liquid must differ very little from ambient temperature (at most, by 5 °C).5.2. Densimetry using a hydrostatic balance

5.2.1. Apparatus

5.2.1.1. The hydrostatic balance is used as described in the chapter 'Density and specific gravity'.5.2.2. Procedure

The apparent density of the distillate at t °C is measured as described in the chapter 'Density and specific gravity', section 5.2.2.
5.2.3. Expression of results

Find the alcoholic strength at 20 °C by following the method described in 4.3.1, using Table I if the float is of Pyrex glass and Table III if it is of ordinary glass.5.3.Refractometry

5.3.1. Apparatus

5.3.1.1. Refractometer enabling refractive indices to be measured in the range 1,330 to 1,346. Depending on the type of equipment, measurements are made:- either at 20 °C with a suitable instrument,- or at ambient temperature t °C by an instrument fitted with a thermometer enabling the temperature to be determined to within at least 0,05 °C. A table giving temperature corrections will be provided with the instrument.5.3.2. Procedure

The refractive index of the wine distillate obtained as in 3.3 above is measured by following the procedure prescribed for the type of instrument used.5.3.3. Expression of results

Table IV is used to find the alcoholic strength corresponding to the refractive index at 20 °C.

Note:Table IV gives the alcoholic strengths corresponding to refractive indices for both pure alcohol-water mixtures and for wine distillates. In the case of wine distillates, it takes into account the presence of impurities in the distillate (mainly higher alcohols). The presence of methanol lowers the refractive index and thus the alcoholic strength.
6. EXAMPLE OF THE CALCULATION OF THE ALCOHOLIC STRENGTH OF A WINE

6.1. Measurement by pycnometer on a twin-pan balance

6.1.1. The constants of the pycnometer have been determined and calculated as described in chapter 1, 'Density and specific gravity', section 6.1.1.6.1.2. Weighing of pycnometer filled with distillateNumeric examplet °C = 18,90 °C
Tare = pycnometer + distillate att °C + p" t °C corrected = 18,70 °C
p" = 2,8074 g

p + m p" = mass of distillate at t °C 105,0698 2,8074 = 102,2624 g

Apparent density at t °C
6.1.3. Calculation of alcoholic strength

Refer to the table of apparent densities of water-alcohol mixtures at different temperatures, as indicated above
On the line 18 °C of the table of apparent densities, the smallest density greater than the observed density of 0,983076 is 0,98398 in column 11 % vol
The density at 18 °C is: (98307,6 + 0,7 × 22) 10 5 = 0,98323
0,98398 0,98323 = 0,00075
The decimal portion of the % vol of alcoholic strength is 75/114 = 0,65
The alcoholic strength is 11,65 % vol6.2. Measurement by pycnometer on a single pan balance

6.2.1. The constants of the pycnometer have been determined and calculated as described in chapter 1, 'Density and specific gravity', section 6.2.1.

6.2.2. Weighing of the pycnometer filled withdistillate

Weight of tare bottle at the time of
measurement in grams: T1 = 171,9178Pycnometer filled with distillate
at 20,50 °C in grams: P2 = 167,8438Variation in the buoyancy of air: dT = 171,9178 171,9160 = +0,0018Mass of the distillate at 20,50 °C: Lt = 167,8438 (67,6695 + 0,0018)

= 100,1725Apparent density of the distillate:6.2.3. Calculation of alcoholic strength

Refer to the table of apparent densities of water-alcohol mixtures at different temperatures, as indicated above
On the line 20 °C of the table of apparent densities, the smallest density greater than observed density of 0,983825 is 0,98471 in column 10 % vol
The density at 20 °C is: (98382,5 + 0,5 × 24) 10 5 = 0,983945
0,98471 0,983945 = 0,000765
The decimal portion of the % vol of alcoholic strength is 76,5/119 = 0,64
The alcoholic strength is 10,64 % vol
FORMULA FROM WHICH TABLES OF ALCOHOLIC STRENGHTS OF ETHANOL-WATER MIXTURES ARE CALCULATEDThe density r in kilograms per metre cubed (kg/m³) of an ethanol-water mixture at temperature t in degrees Celsius is given by the formula below as a function of:

- the alcoholic strength by weight p expressed as a decimal (6),- the temperature t in °C (EIPT 68),- the numerical coefficients below.The formula is valid for temperatures between 20 and +40 °C.

Numerical coefficients in the formula

TABLE I INTERNATIONAL ALCOHOLIC STRENGTH AT 20 °CTable of apparent densities of ethanol-water mixtures - Pyrex pycnometerDensities at t °C corrected for air buoyancy

TABLE II INTERNATIONAL ALCOHOLIC STRENGTH AT 20 °CTable of corrections to be applied to the apparent alcoholic strength to correct for the effect of temperatureAdd or subtract from the apparent alcoholic strength at t °C (ordinary glass alcoholmeter) the correction indicated below

TABLE III INTERNATIONAL ALCOHOLIC STRENGTH AT 20 °CTable of apparent densities of ethanol-water mixtures - Ordinary glass apparatusDensities at t °C corrected for air buoyancy

TABLE IV Table giving the refractive indices of pure ethanol-water mixtures and distillates at 20 °C and the corresponding alcoholic strengths at 20 °C

4. TOTAL DRY EXTRACT

Total dry matter1. DEFINITION

The total dry extract or the total dry matter includes all matter which is non-volatile under specified physical conditions. These physical conditions must be such that the matter forming the extract undergoes as little alteration as possible while the test is being carried out.

The sugar-free extract is the difference between the total dry extract and the total sugars.

The reduced extract is the difference between the total dry extract and the total sugars in excess of 1 g/l, potassium sulphate in excess of 1 g/l, any mannitol present and any other chemical substances which may have been added to the wine.

The residual extract is the sugar-free extract less the fixed acidity expressed as tartaric acid.

The extract is expressed in grams per litre and it should be determined to within the nearest 0,5 g.2. PRINCIPLE OF THE METHOD

Single method: measurement by a densimeter

The total dry extract is calculated indirectly from the specific gravity of the must and, for wine, from the specific gravity of the alcohol-free wine.

This dry extract is expressed in terms of the quantity of sucrose which, when dissolved in water and made up to a volume of one litre, gives a solution of the same specific gravity as the must or the alcohol-free wine. This quantity is shown in Table I.3. METHOD OF CALCULATION

The 20/20 specific gravity drof the 'alcohol-free wine' is calculated using the following formula:dr = dv da + 1,000where:dv = specific gravity of the wine at 20 °C (corrected for volatile acidity) (7),da = specific gravity at 20 °C of a water-alcohol mixture of the same alcoholic strength as the wine.

dr may also be calculated from the densities at 20 °C, rv of the wine and ra of the water-alcohol mixture of the same alcoholic strength by the formula:dr = 1,0018 (rv ra) + 1,000where the coefficient 1,0018 approximates to 1 when rv is below 1,05, which is most often the case.4. EXPRESSION OF RESULTS

Table I should be used for calculating the total dry extract in g/l from the 20/20 specific gravity drof the alcohol-free wine or from the specific gravity d20 °C
20 °C of the must.

The total dry extract is expressed in g/l to one decimal place.

TABLE I for the calculation of the content total dry extract (g/l)

Interpolation table
5. REDUCING SUGARS 1. DEFINITION

Reducing sugars comprise all the sugars exhibiting ketonic and aldehydic functions and are determined by their reducing action on an alkaline solution of a copper salt.2. PRINCIPLE OF THE METHODS

2.1. Clarification

2.1.1. Reference method:after neutralization and removal of alcohol, the wine is passed through an ion exchange column in which its anions are exchanged for acetate ions, followed by clarification with neutral lead acetate.

2.1.2. Usual methods: the wine is treated with one of the following reagents:

2.1.2.1. Neutral lead acetate;

2.1.2.2. Zinc 2-hexacyanoferrate.2.2. Determination

2.2.1. Single method: the clarified wine or must is reacted with a specific quantity of an alkaline copper salt solution and the excess copper ions are then determined iodometrically.3. CLARIFICATION

The sugar content of the liquid in which sugar is to be determined must lie between 0,5 and 5 g/l.

Dry wines should not be diluted during clarification; sweet wines should be diluted during clarification in order to bring the sugar level to within the limits prescribed in the following table:

DescriptionSugar content (g/l)DensityDilution (%)Musts and mistelles>125>1,0381Sweet wines, whether fortified or not25 to 1251,005 to 1,0384Semi-sweet wines5 to 250,997 to 1,00520Dry wines< 5< 0,997No dilution3.1. Reference method

3.1.1. Reagents

3.1.1.1. 1 M solution hydrochloric acid (HCl);

3.1.1.2. 1 M solution sodium hydroxide (NaOH);

3.1.1.3. 4 M solution acetic acid (CH3COOH);

3.1.1.4. 2 M solution sodium hydroxide (NaOH);

3.1.1.5. Anion exchange resin (Dowex 3 (20-50 mesh) or equivalent resin).

Preparation of the anion exchange resin column

Place a small plug of glass wool and 15 ml of the anion exchange resin (3.1.1.5) in the bottom of the burette.

Before the resin is used, subject it to two complete cycles of regeneration by passing alternately the 1 M solutions of hydrochloric acid (3.1.1.1) and sodium hydroxide (3.1.1.2) through it. After rinsing with 50 ml of distilled water, transfer the resin to a beaker, add 50 ml of the 4 M solution of acetic acid (3.1.1.3) and stir for five minutes. Refill the burette with resin and pour 100 ml of the 4 M acetic acid solution (3.1.1.3) through the column. (It is preferable to have a stock of the resin stored in a bottle filled with this 4 M acetic acid solution.) Wash the column with distilled water until the effluent is neutral.

Regeneration of the resin
Pour 150 ml of a 2 M sodium hydroxide solution through the resin to remove acids and most of the pigments fixed to the resin. Rinse with 100 ml of water, and then pour 100 ml of 4 M acetic acid solution through it. Wash the column until the effluent is neutral.

3.1.1.6. Neutral lead acetate solution (approximately saturated)Neutral lead acetate [Pb (CH3 COO)2 · 3 H2O], 250 g;
very hot water to 500 ml;
stir until dissolved.

3.1.1.7. Calcium carbonate (Ca CO3)3.1.2. Procedure

3.1.2.1. Dry wines

Place 50 ml of the wine in a beaker having a diameter of about 10 to 12 cm together with ½ (n 0,5) ml of 1 M sodium hydroxide solution (3.1.1.2) (n being the volume of 0,1 M sodium hydroxide solution used for determining total acidity in 10 ml of wine), and evaporate over a boiling water bath in a stream of hot air until the liquid is reduced to about 20 ml.

Pour this liquid through an anion exchange resin column in acetate form (3.1.1.5) at a rate of 3 ml every two minutes. Collect the effluent in a 100 ml volumetric flask. Wash the vessel and column six times using 10 ml of distilled water each time. Stirring all the time, add 2,5 ml of saturated lead acetate solution (3.1.1.6) and 0,5 g of calcium carbonate (3.1.1.7) to the effluent: shake several times and allow to stand for at least 15 minutes. Make up to the mark with water. Filter.

1 ml of this filtrate corresponds to 0,5 ml of wine.

3.1.2.2. Musts, mistelles, sweet and semi-sweet wines:

The dilutions below are given for guidance.

1. Musts and mistelles: prepare a 10 % solution of the liquid to be analysed and take 10 ml of the diluted sample.

2. Sweet wines, whether fortified or not,having a density between 1,005 and 1,038: prepare a 20 % solution of the liquid to be analysed and take 20 ml of the diluted sample.

3. Semi-sweet wines having a density at 20 °C between 0,997 and 1,005: take 20 ml of the undiluted wine.Allow the abovementioned volume of wine or must to flow through an anion

exchange column in acetate form at a rate of 3 ml every two minutes. Collect the effluent in a 100 ml volumetric flask, and rinse the column with water until about 90 ml of the effluent is obtained. Add 0,5 g calcium carbonate and 1 ml saturated lead acetate solution to the effluent. Stir and allow to stand for 15 minutes, stirring occasionally. Make up to the mark with water. Filter.

In case:1. 1 ml of filtrate corresponds to 0,01 ml of must or mistelle.2. 1 ml of filtrate corresponds to 0,04 ml of sweet wine.3. 1 ml of filtrate corresponds to 0,20 ml of semi-sweet wine.
3.2. Usual methods

3.2.1. Clarification by neutral lead acetate

3.2.1.1. Reagents

Solution of neutral lead acetate (approximately saturated) (see 3.1.1.6).Calcium carbonate.3.2.1.2. Procedure

3.2.1.2.1. Dry wines: place 50 ml of the wine in a 100 ml volumetric flask; add ½ (n 0,5) ml of a 1 M solution of sodium hydroxide (3.1.1.2) (where n is the volume of a 0,1 M solution of sodium hydroxide used to determine the total acidity in 10 ml of wine). Add, while stirring, 2,5 ml of saturated lead acetate solution (3.1.1.6) and 0,5 g calcium carbonate (3.1.1.7). Shake several times and allow to stand for at least 15 minutes. Make up to the mark with water. Filter.1 ml of the filtrate corresponds to 0,5 ml of the wine.3.2.1.2.2. Musts, mistelles, sweet and semi-sweet wines: into a 100 ml volumetric flask, place the following volumes of wine (or must or mistelle), the dilutions being given for guidance:

1. Musts and mistelles: prepare a 10 % solution of the liquid to be analysed and take 10 ml of the diluted sample.

2. Sweet wines, whether fortified or not, having a density between 1,005 and 1,038: prepare a 20 % solution of the liquid to be analysed and take 20 ml of the diluted sample.

3. Semi-sweet wineshaving a density between 0,997 and 1,005: take 20 ml of the undiluted wine.Add 0,5 g calcium carbonate, about 60 ml water and 0,5, 1 or 2 ml of saturated lead acetate solution. Stir and leave to stand for at least 15 minutes, stirring occasionally. Make up to the mark with water. Filter.

In case:1. 1 ml of filtrate corresponds to 0,01 ml of must or mistelle.2. 1 ml of filtrate corresponds to 0,04 ml of sweet wine.3. 1 ml of filtrate corresponds to 0,20 ml of semi-sweet wine.3.2.2. Clarification by zinc 2-hexacyanoferrate

This clarification process should be used only for white wines, lightly coloured sweet wines and musts.3.2.2.1. Reagents

3.2.2.1.1. Solution I, potassium 2-hexacyanoferrate:
Potassium 2-hexacyanoferrate ($K_4 Fe(CN)_6 \cdot 3 H_2O$), 150 g;
Water to 1 000 ml.

3.2.2.1.2. Solution II, zinc sulphate:
Zinc sulphate ($ZnSO_4 \cdot 7 H_2O$), 300 g;
Water to 1 000 ml.
3.2.2.2. Procedure

Into a 100 ml volumetric flask, place the following volumes of wine (or must or mistelle), the dilutions being given for guidance:

1.Musts and mistelles: prepare a 10 % solution of the liquid to be analysed and take 10 ml of the diluted sample.

2. Sweet wines, whether fortified or not, having a density between 1,005 and 1,038: prepare a 20 % solution of the liquid to be analysed and take 20 ml of the diluted sample.

3. Semi-sweet wineshaving a density between 0,997 and 1,005: take 20 ml of the undiluted wine.

4. Dry wines: take 50 ml of undiluted wine.Add 5 ml of solution I, potassium 2-hexacyanoferrate (3.2.2.1.1), and 5 ml of solution II, zinc sulphate (3.2.2.1.2). Mix. Make up to the mark with water. Wait 10 minutes. Filter.

In case:1. 1 ml of filtrate corresponds to 0,01 ml of must or mistelle.2. 1 ml of filtrate corresponds to 0,04 ml of sweet wine.3. 1 ml of filtrate corresponds to 0,20 ml of semi-sweet wine.4. 1 ml of filtrate corresponds to 0,50 ml of dry wine.
4. DETERMINATION OF SUGARS

4.1. Reagents

4.1.1 Alkaline copper salt solution:copper sulphate, pure, $CuSO4 \cdot 5H2O$ 25 g
citric acid ($C6H8O7 \cdot H2O$) 50 g
crystalline sodium carbonate, $Na2CO3 \cdot 10H2O$ 388 g
water to 1 000 mlDissolve the copper sulphate in 100 ml of water, the citric acid in 300 ml of water and the sodium carbonate in 300 to 400 ml of hot water. Mix the citric acid and sodium carbonate solutions. Add the copper sulphate solution and make up to one litre.4.1.2. 30 % potassium iodide solution:potassium iodide (KI) 30 g
water to 100 mlStore in a coloured glass bottle.4.1.3. 25 % sulphuric acid:concentrated sulphuric acid, ($H2SO4$) r20 = 1,84 g/ml 25 g
water to 100 mlAdd the acid slowly to the water, allow to cool and make up to 100 ml with water.4.1.4. 5 g/l starch solution:

Mix 5 g of starch in with about 500 ml of water. Bring to the boil, stirring all the time, and boil for 10 minutes. Add 200 g of sodium chloride (NaCl). Allow to cool and then make up to one litre with water.Sodium thiosulphate, 0,1 M solutionInvert sugar solution, 5 g/l, to be used for checking the method of determination:

Place the following into a 200 ml volumetric flask:pure dry sucrose ($C12H22O11$) 4,75 g
water, approximately 100 ml
concentrated hydrochloric acid (HCl) (r20 = 1,16 - 1,19 g/ml) 5 mlHeat the flask in a water-bath maintained at 60 °C until the temperature of the solution reaches 50 °C; then keep the flask and solution at 50 °C for 15 minutes. Allow the flask to cool naturally for 30 minutes and then immerse it in a cold water-bath. Transfer the solution to a one-litre volumetric flask and make up to one litre. This solution keeps satisfactorily for a month. When it is to be used, neutralize the test sample (the solution being approximately 0,06 M acid) with sodium hydroxide solution.4.2. Procedure

Mix 25 ml of the alkaline copper salt solution, 15 ml water and 10 ml of the clarified solution in a 300 ml conical flask. This volume of sugar solution must not contain more than 60 mg of invert sugar.

Add a few small pieces of pumice stone. Fit a reflux condenser to the flask and bring the mixture to the boil within two minutes. Keep the mixture boiling for exactly 10 minutes.

Cool the flask immediately in cold running water. When completely cool, add 10 ml of 30 % potassium iodide solution (4.1.2), 25 ml of 25 % sulphuric acid (4.1.3) and 2 ml of starch solution (4.1.4).

Titrate with 0,1 M sodium thiosulphate solution (4.1.5) Let nbe the number of ml used.

Also carry out a blank titration in which the 10 ml of sugar solution is replaced by 10 ml of distilled water. Let n2 be the number of ml of sodium thiosulphate used.4.3. Expression of results

4.3.1. Calculations

The quantity of sugar, expressed as invert sugar, contained in the test sample is given in the table below as a function of the number (n2 - n) of ml of sodium thiosulphate used.

The sugar content of the wine is to be expressed in grams of invert sugar per litre to one decimal place, account being taken of the dilution made during clarification and of the volume of the test sample.4.3.2. Repeatability

r = 0,015 xixi = concentration of inverted sugar in g/l per sample4.3.3. Reproducibility

R = 0,058 xixi = concentration of inverted sugar in g/l per sample

6. SUCROSE 1. PRINCIPLE OF METHODS

II. For qualitative testing by thin-layer chromatography: sucrose is separated from other sugars using thin-layer chromatography on plate coated with cellulose. The developing agent is urea-hydrochloric acid at 105 ° C.

II. For testing and determination by high-performance liquid chromatography: the sucrose is separated in a column of alkylamine-bonded silica and detected by refractometry. The result is quantified by reference to an external standard analysed under the same conditions.

Note: Authentication of a must or of a wine may be checked by the method using NMR of deuterium described for detecting the enrichment of musts, rectified concentrated musts and wines.For testing and determination of sucrose, chromatography in gaseous phase may also be used, as described in chapter 42, point (f).2. QUALITATIVE TESTING BY THIN-LAYER CHROMATOGRAPHY

2.1. Equipment

2.1.1. Chromatograhic plates covered with a desired thickness of cellulose powder (e.g. MN 300) (20 × 20).

2.1.2. Chromatography tank.

2.1.3. Micrometric syringe or micropipette.

2.1.4. Oven with regulation to 105 ± 2 °C.2.2. Reagents

2.2.1. Decolourizing charcoal.

2.2.2. Mobile phase:Dichloromethane - glacial acetic acid (p20 - 1,05 g/ml)- ethanol - methanol - water (50:25:9:6:10).

2.2.3. Developing agentUrea 5 g
Hydrochloric acid 2 M 20 ml
Ethanol 100 ml

2.2.4. Reference solutionsGlucose 35 g
Fructose 35 g
Sucrose 0,5 g
Distilled water1 000 ml2.3. Procedure

2.3.1. Preparation of sample

When the must or wine is strongly coloured, decolourize it by treating it with activated charcoal.

For rectified concentrated musts, use the solution with a sugar concentration of 25 % by mass (25 ° Brix) prepared as described in the chapter 'pH of wine and must', section 4.1.2, and dilute it with water to a quarter of its concentration by making 25 ml up to 100 ml in a volumetric flask.2.3.2. Obtaining the chromatogram

Place on a parallel line 2,5 cm from the bottom edge of the plate:- 10 µl of sample- 10 µl of standard.

Place the plate in the tank, previously saturated with the vapour from the mobile phase. Allow the mobile phase to migrate to within 1 cm of the top of the plate. Remove the plate and dry it in a current of warm air. Repeat the migration two more times, drying the plate each time. Spray the plate uniformly with 15 ml of colouring agent and place in the oven at 105 °C for approximately five minutes.2.4. Results

Saccharose and fructose appear as a deep blue spot on a white background: glucose gives a less intense green spot.3. TESTING AND DETERMINATION BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

The chromatographic conditions are given for guidance3.1. Equipment

3.1.1. High-performance liquid chromatograph equipped with:

1. 10 µl loop injector,2. a detector: a differential refractometer or an interferometer refractometer,3. an alkylamine-bonded silica column (length 25 cm, internal diameter 4 mm),4. a guard column filled with the same phase,5. an arrangement for insulating the guard column and analytical columns or for maintaining their temperature (30 ° C),6. a recorder and, if required, an integrator,7. mobile phase flow rate: 1 ml/min.3.1.2. Arrangement for membrane filtration (0,45 µm).3.2. Reagents

3.2.1. Doubly distilled water.

3.2.2. HPLC quality acetonitrile (CH3CN).

3.2.3. Mobile phase: acetonitrile-water, previously subjected to membrane filtration (0,45 µm), (80:20 v/v).This mobile phase must be outgassed before being used.

3.2.4. Standard solution: 1,2 g/l aqueous sucrose solution. Filter using a 0,45 µm membrane filter.
3.3. Procedure

3.3.1. Preparation of sample:

- For wines and musts: filter using a 0,45 µm membrane filter.- For rectified concentrated musts: use the solution obtained by diluting the rectified concentrated must to 40 % (m/v) as described in the chapter 'Total acidity', section 5.1.2., and filtering it using a 0,45 µm membrane filter.3.3.2.Chromatographic determination

Inject in turn into the chromatograph 10 µl of the standard solution and 10 µm of the sample prepared as described in 3.3.1. Repeat these injections in the same order.Record the chromatogram.The retention time of the sucrose is approximately 10 minutes.3.4. Calculations

For the calculation, use the average of two results for the standard solution and the sample.3.4.1. For wines and musts:calculate the concentration in g/l.3.4.2. For rectified concentrated musts: let C be the sucrose concentration in g/l of the 40 % (m/v) solution of rectified concentrated must. The sucrose concentration in g/kg of the rectified concentrated must is then: 2,5C.3.5. Expression of results

The sucrose concentration in wines, musts and rectified concentrated musts is expressed in grams per litre for wines and musts and in grams per kilogram for rectified concentrated musts, each to one place of decimals.

7. GLUCOSE AND FRUCTOSE 1. DEFINITION

Glucose and fructose may be determined individually by an enzymatic method, with the sole aim of calculating the glucose/fructose ratio.2. PRINCIPLE OF THE METHOD

Glucose and fructose are phosphorylated by adenosine triphosphate (ATP) during an enzymatic reaction catalysed by hexokinase (HK), and produce glucose 6-phosphate (G6P) and fructose 6-phosphate (F6P):

glucose + ATP G6P + ADP

fructose + ATP F6P + ADP

The glucose 6-phosphate is first oxidized to gluconate 6-phosphate by nicotinamide adenine dinucleotide phosphate (NADP) in the presence of the enzyme glucose 6-phosphate dehydrogenase (G6PDH). The quantity of reduced nicotinamide adenine dinucleotide phosphate (NADPH) produced corresponds to that of glucose 6-phosphate and thus to that of glucose.

G6P + NADP+ gluconate 6-phosphate + NADPH + H+

The reduced nicotinamide adenine dinucleotide phosphate is determined from its absorption at 340 nm.

At the end of this reaction, the fructose 6-phosphate is converted into glucose 6-phosphate by the action of phosphoglucose isomerase (PGI):

The glucose 6-phosphate again reacts with the nicotinamide adenine dinucleotide phosphate to give gluconate 6-phosphate and reduced nicotinamide adenine dinucleotide phosphate, and the latter is then determined.3. APPARATUS

- A spectrophotometer enabling measurements to be made at 340 nm, the wavelength at which absorption by NADPH is at a maximum. Absolute measurements are involved (i.e. calibration plots are not used but standardization is made using the extinction coefficient of NADPH), so that the wavelength scales of and absorbence values obtained from the apparatus must be checked.If not available, a spectrophotometer using a source with a discontinuous spectrum which enables measurements to be made at 334 nm or at 365 nm may be used.- Glass cells with optical path lengths of 1 cm or single-use cells.- Pipettes for use with enzymatic test solutions, 0,02, 0,05, 0,1, 0,2 ml.4. REAGENTS

4.1. Solution 1:buffer solution (0,3 M triethanolamine, pH 7,6, 4 × 10 3 M in Mg 2+): dissolve 11,2 g triethanolamine hydrochloride ((C2H5)3N · HCl) and 0,2 g MgSO4 · 7H2O in 150 ml of doubly distilled water, add about 4 ml of 5 M sodium hydroxide (NaOH) solution to obtain a pH value of 7,6 and make up to 200 ml.

This buffer solution may be kept for four weeks at +4 °C.

4.2. Solution 2: nicotinamide adenine dinucleotide phosphate solution (about 11,5 × 10 3 M): dissolve 50 mg disodium nicotinamide adenine dinucleotide phosphate in 5 ml of doubly distilled water.

This solution may be kept for four weeks at +4 °C.

4.3. Solution 3: adenosine 52-triphosphate solution (about 81 × 10 3 M): dissolve 250 mg disodium adenosine 52-triphosphate and 250 mg sodium hydrogencarbonate (NaHCO3) in 5 ml of doubly distilled water.

This solution may be kept for four weeks at +4 °C.

4.4. Solution 4: hexokinase/glucose 6-phosphate dehydrogenase: mix 0,5 ml hexokinase (2 mg of protein/ml or 280 U/ml) with 0,5 ml glucose 6-phosphate dehydrogenase (1 mg of protein/ml).

This mixture may be kept for a year at about +4 °C.

4.5. Solution 5: phosphoglucose isomerase (2 mg of protein/ml or 700 U/ml). The suspension is used undiluted.

This may be kept for a year at about +4 °C.

Note:All solutions used above are available commercially.5. PROCEDURE

5.1. Preparation of sample

Depending on the estimated amount of glucose + fructose per litre, dilute the sample as follows:

Measurement
at 340 and 334 nmMeasurement
at 365 nmDilution with waterDilution factor Fup to 0,4 g/l 0,8 g/l--up to 4,0 g/l 8,0 g/l1 + 9 10up to 10,0 g/l20,0 g/l1 + 24 25up to 20,0 g/l40,0 g/l1 + 49 50up to 40,0 g/l80,0 g/l1 + 99 100above 40,0 g/l80,0 g/l1 + 9991 000
5.2. Determination

With the spectrophotometer adjusted to the 340 nm wavelength, make measurements using air (no cell in the optical path) or water as reference.Temperature between 20 and 25 °C.Into two cells with 1 cm optical paths, place the following:

Reference cell Sample cellSolution 1 (4.1) (taken to 20 °C) 2,50 ml 2,50 ml
Solution 2 (4.2) 0,10 ml0,10 ml
Solution 3 (4.3) 0,10 ml 0,10 ml
Sample to be measured 0,20 ml
Doubly distilled water 0,20 ml

Mix, and after about three minutes read off the absorbence of the solutions (A1). Start the reaction by adding:Solution 4 (4.4) 0,02 ml 0,02 ml

Mix; wait 15 minutes; read off the absorbence and check that the reaction has stopped after a further two minutes (A2).

Add immediately:Solution 5 (4.5) 0,02 ml 0,02 ml

Mix; read off the absorbence after 10 minutes and check that the reaction has stopped after a further two minutes (A3).

Calculate the differences in the absorbences:A2 A1 corresponding to glucose,A3 A2 corresponding to fructose,for the reference and sample cells.

Calculate the differences in absorbence for the reference cell (DAR) and the sample cell (DAS) and then obtain:for glucose: DAG = DAS DARfor fructose: DAF = DAS DAR

Note:The time needed for the completion of enzyme activity may vary from one batch to another. The above value is given only for guidance and it is recommended that it be determined for each batch.5.3.Expression of results

5.3.1. Calculation

The general formula for calculating the concentrations is:where V = volume of the test solution (ml)v = volume of the sample (ml)M = molecular mass of the substance to be determinedd = optical path in the cell (cm)e = absorption coefficient of NADPH at 340 nm (= 6,3 mM 1 × l × cm 1)and V = 2,92 ml for the determination of glucoseV = 2,94 ml for the determination of fructosev = 0,20 mlM = 180d = 1

so that:For glucose: C (g/l) = 0,417 DAG
For fructose: C (g/l) = 0,420 DAF

If the sample was diluted during its preparation, multiply the result by the dilution factor F.

Note:If the measurements are made at 334 or 365 nm, then the following expressions are obtained:- measurement at 334 nm: e = 6,2 (mmole 1 × 1 × cm 1)For glucose: C (g/l) = 0,425 DAG
For fructose: C (g/l) = 0,428 DAF- measurement at 365 nm: e = 3,4 (mmole × 1 1 × cm 1)For glucose: C (g/l) = 0,773 DAG
For fructose: C (g/l) = 0,778 DAF5.3.2. Repeatability (r)

r = 0,056 xi5.3.3. Reproducibility (R)

R = 0,12 + 0,076 xi
xi = concentration of glucose or fructose in g/l.

8. DETECTING ENRICHMENT OF GRAPE MUSTS, CONCENTRATED GRAPE MUSTS, RECTIFIED CONCENTRATED GRAPE MUSTS AND WINES BY APPLICATION OF NUCLEAR MAGNETIC RESONANCE OF DEUTERIUM (SNIF-NMR/RMN-FINS) 1. DEFINITION

The deuterium contained in the sugars and the water in grape must will be redistributed after fermentation in molecules I, II, III and IV of the wine:CH2D CH2 OH CH3 CHD OH
I IICH3 CH2 OD HOD
III IV

The addition of exogenous sugar (sugaring in the dry) before the must ferments will have an effect on the distribution of the deuterium.

As compared with the figures for parameters for a natural control wine from the same region, the enrichment of an exogenous sugar will lead to the following variations:

Parameters
Wine(D/H)I(D/H)II(D/H)QWR- Natural - Enriched: - beet sugar - cane sugar - maize sugar

(D/H)I : Isotope ratio associated with molecule I(D/H)II : Isotope ratio associated with molecule II (D/H)QW : Isotope ratio of the water in the wine

R = 2(D/H)II/(D/H)I expresses the relative distribution of deuterium in molecules I and II; R is measured directly from the h-intensities of the signals and thenR = 3hII/hI.

(D/H)I mainly characterizes the vegetable species which synthesized the sugar and to a lesser extent the geographical location of the place of harvest (type of water used during photosynthesis).

(D/H)II represents the climatology of the place of production of the grapes (type of rainwater and weather conditions) and to a lesser extent the sugar concentration of the original must.

(D/H)QW represents the climatology of the place of production and the sugar content of the original must.2. PRINCIPLE

The parameters defined above (R,(D/H)I, (D/H)II) are determined by nuclear magnetic resonance of the deuterium in the ethanol extracted from the wine or from the fermentation products of the must, the concentrated must or the rectified concentrated must obtained under given conditions; they may be supplemented by determining the isotope ratio of the water extracted from the wine, (D/H)QW and by determining the ratio13C/12C in the ethanol.

Pending the creation of a Community data bank proceed as follows:In the case of wines, control samples taken in the regions must be accompanied by natural control samples (at least three) of the same origin (geographical place and vintage); three series of such samples are to be taken.In the case of musts, concentrated musts and rectified concentrated musts, three series of control samples are to be made up of natural musts of the same origin (geographical place and vintage).

For checks on products prepared within their own territory and pending the establishment of a Community data bank, the Member States may on a transitional basis use a national data bank.3. PREPARATION OF THE SAMPLE FOR ANALYSIS

3.1. Extraction of ethanol and water in the wine

Note:Any method for ethanol extraction can be used as long as 98 to 98,5 % of the total alcohol in the wine is recovered in a distillate which contains 92 to 93 % mas (95 % vol).3.1.1. Apparatus and reagents

Apparatus for extracting ethanol (Figure 1) comprising:- electric heating mantle with voltage regulator,- one-litre round-bottom flask with ground glass neck joint,- Cadiot column with rotating band (moving part in Teflon),- 125 ml conical flasks with ground glass neck joints,- 125 and 60 ml bottles with plastic stoppers.Reagents for the determination of water by the Karl Fischer method (e.g. Merck 9241 and 9243).3.1.2. Procedure

3.1.2.1. Determine the alcoholic strength of the wine (tv) to better than the nearest 0,05 % vol.3.1.2.2. Extraction of the ethanol

Introduce a homogeneous sample of 500 ml of wine of alcoholic strength tv into the flask in the distillation apparatus with a constant reflux ratio of about 0,9. Place a 125 ml ground conical flask, calibrated beforehand, to receive the distillate. Collect the boiling liquid between 78,0 and 78,2 °C, i.e. approximately 40 to 60 ml. If the temperature exceeds 78,5 °C, discontinue collection for five minutes.

When the temperature returns to 78 °C, recommence collecting the distillate until 78,5 °C and repeat this operation until the temperature, after discontinuing collection and operating within a closed circuit, remains constant. Complete distillation lasts approximately five hours. This procedure enables between 98 and 98,5 % of the total alcohol in the wine to be recovered in a distillate with a strength of between 92 and 93 % mas (95 % vol), a strength for which the NMR conditions described in section 4 have been established.

The ethanol collected is weighed.

A homogeneous 60 ml sample of the residues is kept in a 60 ml flask and represents the water in the wine. Its isotope ratio may be determined if required.

Note:If a spectrometer fitted with a 10 mm probe is available (cf. section 4), a homogeneous test sample of 300 ml of wine is sufficient.3.1.2.3. Determination of the alcoholic strength of the alcohol extracted

The water content (p2 g) is determined by the Karl Fischer method using a sample of about 0,5 ml of alcohol of exactly known mass p.

Figure 1Distillation apparatus for the extraction of ethanol

The strength by mass of the alcohol is given by3.2. Fermentation of musts, concentrated musts and rectified concentrated musts

3.2.1. Apparatus and reagents

Tartaric acidDIFCO Bacto Yeast Nitrogen Base without amino acidsActive dry yeasts (Saccharomyces cerevisae)

If the isotope ratio of the must is known the yeast can be reactivated prior to use for 15 minutes in a minimum amount of lukewarm non-distilled water, so that the isotope ratio is similar to that of the must.

If the isotope ratio of the must is not known it is better to use fresh/direct.

Fermentation vessel of a capacity of 1,5 litres fitted with a device to keep it airtight and to condense alcohol vapour, since no loss of ethanol during fermentation must be tolerated. The rate of conversion of fermentable sugars into ethanol should be greater than 98 %.3.2.2. Procedure

3.2.2.1. Musts

- Fresh mustsPlace one litre of must, whose concentration of fermentable sugars has been previously determined, in the fermentation vessel. Add 1 g of dry yeast reactivated beforehand. Insert device to keep it airtight. Allow fermentation to proceed at around 20 °C until the sugar is used up. After determining the alcoholic strength of the fermentation product and calculating the rate of conversion of sugars into alcohol, the fermented liquid is centrifuged and distilled to extract the ethanol.

- Musts with fermentation prevented by the addition of sulphur dioxideDe-sulphite a quantity of must slightly in excess of one litre (i.e. 1,2 litre) by bubbling nitrogen through the must in a water bath at 70 to 80 °C under reflux until the total sulphur dioxide content is less than 200 mg/l. Take care to see that the must is not concentrated through evaporation of water by using effective cooling. Place 1 litre of de-sulphited must in the fermentation vessel and continue as described for fresh must.Note:If potassium metabisulphite is used to sulphite the must, 0,25 ml of sulphuric acid (r20 = 1,84 g/ml) per gram of metabisulphite used per litre of must should be added to the must before de-sulphiting.3.2.2.2. Concentrated musts

Place V ml of concentrated must containing a known amount of sugar (approximately 170 g) into the fermentation vessel. Top up to one litre with (1 000 V) ml of water from the normal water supply of same isotope ratio as natural must samples. Add (3.2.1) dry yeasts (1 g) and 3 g of DIFCO Bacto Yeast Nitrogen Base without amino acids. Homogenize and proceed as before.3.2.2.3. Rectified concentrated musts

Proceed as described in 3.2.2.2, topping up to one litre with (1 000 V) ml of water from the normal water supply of same isotope ratio but also containing 3 g dissolved tartaric acid.

Note:Retain 50 ml of sample of must or sulphur dioxide treated must or concentrated must or rectified concentrated must with a view to the possible extraction of the water and the determination of its isotope ratio (D/H)WQ. The extraction of the water contained in the must may be very simply carried out by azotropic distillation using toluene.
3.3. Preparation of alcohol sample for NMR measurement

3.3.1. Reagents

N, N-tetramethyl urea (TMU); use a sample of standard TMU with a given, monitored isotope ratio D/H. This sample may be supplied by:Directorate-General for Science, Research and Development,
Community Bureau of References,
200 rue de la Loi, B-1049 Brussels.3.3.2. Procedure

- 15 mm diameter NMR probe:in a previously weighed bottle, collect 7 ml alcohol obtained as in 3.1.2 and weigh it to the nearest 0,1 mg (mA); then take a 3 ml sample of the internal standard (TMU) and weigh to the nearest 0,1 mg (mst). Homogenize by shaking.

- 10 mm diameter NMR probe:
3,2 ml of alcohol and 1,3 ml TMU are sufficient.Depending on the type of spectrometer and probe used (cf. section 4), add a sufficient quantity of hexafluorobenzene as a field-frequency stabilization substance (lock):

Spectrometer10 mm probe15 mm probe7,05 T150 µl200 µl9,4 T 35 µl 50 µl
3.4. Preparation of a water sample for the NMR measurement, for the purpose of a possible determination of its isotope ratio3.4.1. Reagents

N, N-tetramethyl urea (TMU) : see 3.3.1.
3.4.2. Procedure

Place 3 ml of water obtained as in 3.1.2 or 3.2 (note) into a tared flask and weigh to the nearest 0,1 mg (m2E). Place 4 ml of internal standard (TMU) and weigh to the nearest 0,1 mg (m2st). Homogenize by shaking.

Note:If the laboratory has a mass spectrometer for determining isotope ratios, the measurement may be carried out on this instrument to reduce the load on the NMR spectrometer. It is necessary to standardize the ratio Trv (5.2) for each series of wines examined.4. RECORDING OF ^2H NMR SPECTRA OF THE ALCOHOL AND THE WATER

Determination of isotope parameters.4.1. Apparatus

- NMR spectrometer fitted with a specific 'deuterium' probe tuned to the characteristic frequency Vo of the field Bo (e.g. for Bo = 7,05 T, Vo = 46,05 MHz and for Bo = 9,4 T, Vo = 61,4 MHz) having a proton decoupling channel (B2) and field-frequency stabilization channel (lock) at the fluorine frequency.

The resolution measured on the spectrum, transformed without exponential multiplication (i.e. LB = 0) (Figure 2b) and expressed by the half-width of the methyl and methylene signals of ethanol and the methyl signal of TMU, must be less than 0,5 Hz. The sensitivity, measured with an

exponential multiplying factor LB equal to 2 (Figure 2a) must be greater than or equal to 150 for the methyl signal of ethanol of alcoholic strength 95 % vol (93,5 % mas).

Under these conditions, the confidence interval for the measurement of the signal height, calculated for a 97,5 % probability (one-sided test) and 10 repetitions of the spectrum, is 0,35 %.- Automatic sample changer (possibly)- Data-processing software- 15 mm or 10 mm sample tubes according to spectrometer performance.4.2. Standardization of spectrometer and checks

4.2.1. Standardization

Carry out customary standardization for homogeneity and sensitivity according to the manufacturer's specifications.4.2.2. Checking the validity of the standardization

Use standard ethanols, designated by the letters C, V and B, having isotope concentrations that are different but accurately standardized. They carry the following meaning:- C: alcohol from cane sugar or maize,- V: wine spirit,- B: beet alcohol.These samples are supplied by the Community Bureau of References.

Following the procedure described in 4.3, determine the isotope values of these alcohols, denoting them Cmeas, Vmeas, Bmeas (see 5.3).

Compare them with the given corresponding standard values, denoted by a superscript Cst, Bst, Vst (see 5.3).

Figure 2a²H NMR spectrum of an ethanol from wine with an internal standard (TMU: N, N-tetramethylurea)

Figure 2b²H spectrum of ethanol taken under the same conditions as those of Figure 2a, but without exponential multiplication (LB = 0)

The standard deviation for repeatability obtained on an average of 10 repetitions of each spectrum must be less than 0,01 for the ratio R and less than 0,3 ppm for (D/H)I and (D/H)II.

The average values obtained for the various isotopic parameters (R, (D/H)I, (D/H)II) must be within the corresponding standard deviation of repeatability given for those parameters for the three standard alcohols by the Community Bureau of References. If they are not, carry out the checks again.4.3. Conditions for obtaining NMR spectra

Place a sample of alcohol prepared as in 3.3 (or the water sample, prepared as in 3.4) in a 15-mm or 10-mm tube and introduce it into the probe.

The conditions for obtaining NMR spectra are as follows:- a constant probe temperature (e.g. 302 K); - acquisition time of at least 6,8 s for 1 200 Hz spectral width (16K memory) (i.e. about 20 ppm at 61,4 MHz or 27 ppm at 46,1 MHz); - 90° pulse; - adjustment of acquisition time: its value must be of the same order as the dwell time; - parabolic detection: fix the offset 01 between the OD and CHD reference signals for ethanol and between the HOD and TMU reference signals for water; - determine the value of the decoupling offset 02 from the proton spectrum measured by the decoupling coil on the same tube. Good decoupling is obtained when 02 is located in the middle of the frequency interval existing between the CH3- and CH2- groups. Use the wide band decoupling mode.

For each spectrum, carry out a number of accumulations NS sufficient to obtain the signal-to-noise ratio given in 4.1 and repeat this set of NS accumulations NE = 10 times. The values of NS depend on the types of spectrometer and probe used (cf. section 4). Examples of the possible choices are:

Spectrometer10 mm probe15 mm probe7,05 TNS = 304NS = 2009,4 TNS = 200NS = 128
5.EXPRESSION OF RESULTS

5.1. Ethanol

For each of the 10 spectra (see NMR spectrum for ethanol, Figure 2a), determine:- mst and mA, see 3.3.2.- tD, see 3.1.2.3.- (D/H)st = isotope ratio of internal standard (TMU) indicated on the bottle supplied by the Community Bureau of References.

The use of peak heights instead of peak area, which is less precise, supposes that peak width at half height is identical and is a reasonable approximation if applicable (Figure 2b).5.2. Water

When the isotope ratio of water is determined by NMR from the water-TMU mixture, the following relationship is used:with- m2st and m2E, see 3.4.2.- (D/H)st = isotope ratio of the internal standard (TMU) indicated on the bottle supplied by the Community Bureau of References.

5.3. For each of the isotope parameters, calculate the average of 10 determinations and the confidence interval.

Optional software (e.g. SNIF-NMR) suitable for the spectrometer computer enables such calculations to be carried out on-line.

Note:If, after standardization of the spectrometer, there is a systematic difference between the average values obtained for the characteristic isotopes of the standard alcohols (4.2.2) and the values indicated by the Community Bureau of References, to within the standard deviation, the following corrections may be applied to obtain the true value for any sample X.

The interpolation will be made by taking the values for the standard sample which straddle that of the sample X.

Let (D/H)iXmeas be the measured value and (D/H)iXcorr be the corrected value. This will give:(D/H)iXcorr = (D/H)iBst + a [(D/H)iXmeas (D/H)iBmeas]where

Example:Standard samples supplied and standardized by the Community Bureau of References:(D/H)IVst = 102,0 ppm (D/H)IBst = 91,95 ppmStandard samples measured by the laboratory:(D/H)IVmeas = 102,8 ppm (D/H)IBmeas = 93,0 ppmSuspect uncorrected sample:

(D/H)IXmeas = 100,2 ppma = 1,0255 and (D/H)IXcorr. = 99,3 ppm are calculated.6. INTERPRETATION OF RESULTS

Compare the value RX obtained for the R ratio of the suspect sample with the ratios obtained for the control wines. If RX differs by more than two standard deviations from the average R T value obtained for the control wine, adulteration may be assumed.6.1. Addition of beet sugar, cane sugar or maize glucose

6.1.1. Wines

RX higher than RT: beet sugar is assumed to have been added.
RX less than RT: cane sugar or maize sugar is assumed to have been added.Note that (D/H)XI and (D/H)WQX are increased.Consider (D/H)XI:- Beet sugar is assumed to have been added: (D/H)XI of the suspect sample is lower than (D/H)IT, the average value obtained from the control samples, by more than one standard deviation- Cane sugar or maize sugar is assumed to have been added:
(D/H)IX is greater than (D/H)IT by more than one standard deviation- Calculation of enrichment E expressed in % vol of ethanol:- Addition of beet sugar:where(D/H)IB = isotope ratio for the location I of the beet alcohol; (D/H)IB = 92,5 (8) tV = alcoholic strength of the analysed wine (X).- Addition of cane sugar or maize sugar:
where(D/H)IC = isotope ratio for the location I of the cane sugar or maize sugar; (D/H)IC = 110,5 (9) tV = alcoholic strength of the analysed wine (X)6.1.2.Musts, concentrated musts and rectified concentrated masts

The values of the isotopic parameters for the alcohol extracted as described in 3.1 from the fermented product obtained (3.2) from must, concentrated must and rectified concentrated must are examined according to the instructions given in 6 under 'Interpretation of results' (6.1.1) and compared with the alcohol extracted from the fermentation product of musts.

The enrichment, E % vol, expresses the volume of alcohol added to the fermented product. Knowing the dilution that may have been carried out prior to fermentation (concentrated musts and rectified concentrated musts), assuming that 16,83 g of sugar yield 1 % vol of alcohol, calculate the amount of sugar (mass) added per litre of must, concentrated must or rectified concentrated must.6.2. Addition of a mixture of beet sugar and cane sugar or maize glucose

The isotope ratios (D/H)I and R are changed less than when only one type of sugar is added.

(D/H)II is higher, as is (D/H)WQ.These additions may be confirmed by determining the13C/12C ratio of the ethanol by mass spectrometry; in that case the ratio is higher.

9. ASH CONTENT 1. DEFINITION

The ash content is defined to be all those products remaining after igniting the residue left from evaporation of the wine. The ignition is carried out in such a way that all the cations (excluding the ammonium cation) are converted into carbonates or other anhydrous inorganic salts.2. PRINCIPLE OF THE METHOD

The wine extract is ignited at a temperature between 500 and 550 °C until complete combustion (oxidation) of organic material has been achieved.3. APPARATUS

3.1. boiling water-bath;

3.2. balance sensitive to 0,1 mg;

3.3. hot-plate or infra-red evaporator;

3.4. temperature-controlled electric muffle furnace;

3.5. desiccator;

3.6. flat-bottomed platinum dish 70 mm in diameter and 25 mm in height.4. PROCEDURE

Pipette 20 ml of wine into the previously tared platinum dish (original weight Po g). Evaporate on the boiling water-bath, and heat the residue on the hot-plate at 200 °C or under the infra-red evaporator until carbonization begins. When no more fumes are produced, place the dish in the electric muffle furnace maintained at 525 ± 25 °C. After 15 minutes of carbonization, remove the dish from the furnace, add 5 ml of distilled water, evaporate on the water-bath or under the infra-red evaporator, and again heat the residue to 525 °C for 10 minutes.

If combustion (oxidation) of the carbonized particles is not complete, repeat the operations of washing the carbonized particles, evaporation of water and ignition.

For wines with a high sugar content, it is advantageous to add a few drops of pure vegetable oil to the extract before the first ashing to prevent excessive foaming.

After cooling in the desiccator, the dish is weighed (P1 g).

The weight of the ash in the sample (20 ml) is then P = (P1- Po) g.5. EXPRESSION OF RESULTS

5.1. Method of calculation

The weight P of the ash in grams per litre will be given to two decimal places by the expression: P= 50p

10. ALKALINITY OF THE ASH 1. DEFINITION

The alkalinity of the ash is defined as the sum of cations, other than the ammonium ion, combined with the organic acids in the wine.2. PRINCIPLE OF THE METHOD

The ash is dissolved in a known (excess) amount of a hot standardized acid solution; the excess is determined by titration using methyl orange as an indicator.3. REAGENTS AND APPARATUS

3.1. 0,05 M sulphuric acid solution (H_2SO_4);

3.2. 0,1 M sodium hydroxide solution (NaOH);

3.3. methyl orange, 0,1 % solution in distilled water;

3.4. boiling water-bath.4. PROCEDURE

Add 10 ml of the 0,05 M sulphuric acid solution (3.1) to the ash from 20 ml of wine contained in the platinum dish. Place the dish on the boiling water-bath for about 15 minutes, breaking up and agitating the residue with a glass rod to speed up the dissolution. Add two drops of methyl orange solution and titrate the excess sulphuric acid against 0,1 M sodium hydroxide (3.2) until the colour of the indicator changes to yellow.5. EXPRESSION OF RESULTS

Method of calculation

The alkalinity of the ash, expressed in milliequivalents per litre to one decimal place, is given by

A = 5 (10 n)where n ml is the volume of 0,1 M sodium hydroxide used.

11. CHLORIDES 1. PRINCIPLE

Chlorides are determined directly in the wine by potentiometry using an Ag/AgCl electrode.2. APPARATUS

2.1. pH/mV meter graduated at intervals of at least 2 mV.

2.2. Magnetic stirrer.

2.3. Ag/AcCl electrode with a saturated solution of nitrate potassium as electrolyte.

2.4. Microburette graduated in 1/100 ml.

2.5. Chronometer.3. REAGENTS

3.1. Standard chloride solution: 2,1027 g of potassium chloride, KCl (max. 0,005 % Br), dried before use by leaving in a desiccator for several days, are diluted in distilled water and made up to one litre. 1 ml of this solution contains 1 mg Cl .

3.2. Silver nitrate titrating solution: 4,7912 g of analytical grade silver nitrate, AgNO3 are diluted in a 10 % (v/v) alcohol solution and made up to one litre. 1 ml of this solution corresponds to 1 mg Cl .

3.3. Nitric acid, of at least 65 % purity (r20 = 1,40 g/ml).4. PROCEDURE

4.1. 5,0 ml of standard chloride solution are measured into a 150 ml cylindrical vessel placed on a magnetic stirrer, diluted with distilled water to approximately 100 ml and acidified with 1,0 ml of nitric acid (at least 65 %). After immersing the electrode, titrate by adding the silver nitrate titrating solution with the microburette, with moderate stirring. Begin by adding 1,00 ml for the first 4 ml and read the corresponding millivolt values. Add the next 2 ml in fractions of 0,20 ml. Finally, continue the addition in fractions of 1 ml until a total of 10 ml has been added. After each addition, wait for approximately 30 seconds before reading the corresponding millivolts. Transfer the values thus obtained onto graph paper against the corresponding millilitres of titrating solution and determine the potential of the equivalence point on the basis of the singular point on the curve obtained.

4.2. 5 ml of the standard chloride solution are measured into a 150 ml cylindrical vessel with 95 ml of distilled water and 1 ml of nitric acid (at least 65 %). Immerse the electrode and titre, whilst stirring, until the potential of the equivalence point is obtained. This determination is repeated until a good degree of agreement in the results is obtained. This check must be carried out before each series of measurements of chlorides in the samples.

4.3. 50 ml of wine for analysis are measured into a 150 ml cylindrical vessel. Add 50 ml of distilled water and 1 ml of nitric acid (at least 65 %) and titrate using the procedure describedin 4.2.5. EXPRESSION OF RESULTS

5.1. Calculations

If n represents the number of millilitres of silver nitrate titrating solution, the chloride content in the tested liquid is:

20 × n expressed as milligrams of Cl per litre,0,5633 × n expressed as milliequivalents per litre,32,9 ×n expressed as milligrams of sodium chloride per litre.5.2. Repeatability (r):

r = 1,2 mg Cl per litrer = 0,03 meg per litrer = 2,0 mg NaCl per litre5.3. Reproducibility (R):

R = 4,1 mg Cl per litreR = 0,12 meg per litreR = 6,8 mg NaCl per litre6. Note: For very precise determination.

Refer to the complete titration curve obtained during determination of the test liquid with the silver nitrate solution.

(a) Measure 50 ml of the wine to be analysed into a 150 ml cylindrical vessel. Add 50 ml of distilled water and 1 ml of nitric acid (at least 65 %). Titrate using the silver nitrate solution, adding 0,5 ml at a time and recording the corresponding potential in millivolts. Derive from this first titration the approximate volume of silver nitrate solution required.

(b) Recommence determination in the same conditions. Begin by adding 0,5 ml of titrating solution at a time until the volume added is 1,5 to 2 ml less that the volume determined in (a). Hereafter add 0,2 ml at a time. Continue to add the solution beyond the approximately located equivalence point in a symmetrical manner, i.e. by adding 0,2 ml and then 0,5 ml at a time.

The end point of the measurement and the exact volume of silver nitrate consumed are obtained:- either by drawing the curve and determining the equivalence point,- or by the following calculation:Where:V = volume of titrating solution at equivalence point; V2 = volume of titrating solution before the largest potential change; D Vi = constant volume of the increments of titrating solution, i.e. 0,2 ml; DD El = second difference in potential before the largest potential change; DD E2 = second difference in potential after the largest potential change.

Example:

Volume of AgNO3 titrating solutionE potential in mVDifference D ESecond difference DD E 0204 4 0,2208 0 4 0,4212 2 6 0,6218 0 6 0,8224 0 6 1,0230 2 8 1,2238 4 12 1,4250 10 22 1,6272 22 44 1,8316 10 34 2,0350 8 26 2,2376 6 20 2,4396

In this example, the end point of the titration is between 1,6 and 1,8 ml: the largest potential change (D E = 44 mV) occurs in this interval. The volume of silver nitrate titrating solution consumed to measure the chlorides in the test sample is:

12. SULPHATES 1. PRINCIPLE

1.1. Reference method

Precipitation of barium sulphate and weighing. The barium phosphate precipitated in the same conditions is eliminated by washing the precipitate in hydrochloric acid.

In the case of musts or wine rich in sulphur dioxide, prior de-sulphiting by boiling in an airtight vessel is recommended.1.2. Quick test method

Wines are classified into several categories using the so-called limits method, based on the precipitation of barium sulphate using a barium ion titrant.2. REFERENCE METHOD

2.1. Reagents

2.1.1. 2 M solution of hydrochloric acid.

2.1.2. Barium chloride solution of 200 g/l of $BaCl_2 \cdot 2H_2O$.2.2. Procedure

2.2.1. General procedure:

Measure 40 ml of the analysis sample into a 50 ml centrifuge tube; add 2 ml of 2 M hydrochloric acid and 2 ml of barium chloride solution at 200 g/l. Stir with a glass stirrer; rinse the stirrer with a little distilled water and leave to stand for five minutes. Centrifuge for five minutes, then carefully decant the supernatant liquid.

Next wash the barium sulphate precipitate as follows: add 10 ml of 2 M hydrochloric acid, place the precipitate in suspension and centrifuge for five minutes, then carefully decant the supernatant liquid. Repeat the washing procedure twice in the same conditions using 15 ml distilled water each time.

Quantitatively transfer the precipitate, by rinsing with distilled water, into a tared platinum capsule and place over a water bath at 100 °C until fully evaporated. The dried precipitate is calcined several times briefly over a flame until a white residue is obtained. Leave to cool in a desiccator and weigh.

Let m= the mass in milligrams of barium sulphate obtained.2.2.2. Special procedure: sulphited must and wine with a high sulphur dioxide content.

Beforehand, eliminate the sulphur dioxide.

Measure 25 ml of water and 1 ml of pure hydrochloric acid (r 20 = 1,15 to 1,18 g/ml) into a 500 ml conical flask equipped with a dropping funnel and an outlet tube. Boil the solution to remove the air and introduce 100 ml of wine through the dropping funnel. Continue boiling until the volume of liquid in the flask has been reduced to about 75 ml and quantitatively transfer it, after cooling, to a 100 ml volumetric flask. Make up to mark with water. Determine the sulphates in a 40 ml sample as indicated in 2.2.1.
2.3. Expression of results

2.3.1. Calculations:

The sulphate content, expressed in milligrams per litre of potassium sulphate, K_2SO_4 is:18,67 × m

The sulphate content in musts or wine is expressed in milligrams per litre of potassium sulphate, with no decimal point.2.3.2. Repeatability

up to 1 000 mg/l: r = 27 mg/labout 1 500 mg/l: r = 41 mg/l2.3.3. Reproducibility

up to 1 000 mg/l: R = 51 mg/labout 1 500 mg/l: R = 81 mg/l3. QUICK TEST METHOD

3.1. Reagents

3.1.1. Barium chloride titrating solution

2,804 g of barium chloride, $BaCl_2 \cdot 2H_2O$ and 10 ml of hydrochloric acid (r20 = 1,15 to 1,18 g/ml) are dissolved in enough water to obtain one litre of solution, 1 ml of this solution precipitates a quantity of sulphate ions equivalent to 2 mg of potassium sulphate.

3.1.2. Sulphuric acid (r20 = 1,84 g/ml), 1/10 (m/v) solution.3.2. Procedure

Measure 10 ml of musts or wine into three test tubes; add to No 1: 3,5 ml, to No 2: 5 ml and to No 3: 10 ml of the barium chloride solution. Shake and bring to the boil; leave to stand for one to two hours. The liquid in each tube is decanted, filtered and divided into two parts. To one part, add several drops of the dilute sulphuric acid solution, to the other some drops of the barium chloride solution. Examine each tube and note whether the liquid is clear or cloudy. Interpretation of these observations is given in the table below.

WineBarium chlorideFiltered wine +diluted sulphuric acidbarium chloride solutionFirst test(ml)(ml)cloudyclear103,5(less than 0,7 g K_2SO_4/l)
clear cloudy

(more than 0,7 g K2SO4/l)Second test105cloudyclear(less than 1 g K2SO4/l)
clear cloudy
(more than 1 g K2SO4/l)Third test1010cloudyclear(less than 2 g K2SO4/l)
clear cloudy
(more than 2 g K2SO4/l)

13. TOTAL ACIDITY 1. DEFINITION

The total acidity of the wine is the sum of its titratable acidities when it is titrated to pH 7 against a standard alkaline solution.

Carbon dioxide is not included in the total acidity.2. PRINCIPLE OF THE METHOD

Potentiometric titration or titration with bromothymol blue as an indicator and comparison with an end-point colour standard.3. REAGENTS

3.1. Buffer solution pH 7,0:

- monopotassium phosphate, (KH2PO4) 107,3 g- 1 M sodium hydroxide (NaOH) solution 500 ml- water to1 000 ml

Alternatively, ready made buffer solutions are available commercially.

3.2. 0,1 M sodium hydroxide (NaOH) solution.

3.3. 4 g/l bromothymol blue indicator solution:

- bromothymol blue (C27H28Br2O5S) 4 g- neutral ethanol, 96 % vol 200 mlDissolve and add:- water free of CO2 200 ml- 1 M sodium hydroxide solution sufficient to produce blue-green colour (pH 7) 7,5 ml approximately- water to1 000 ml4. APPARATUS

4.1. Water vacuum pump.

4.2. 500 ml vacuum flask.

4.3. Potentiometer with scale graduated in pH values, and electrodes. The glass electrode must be kept in distilled water. The calomel/saturated potassium chloride electrode must be kept in a saturated potassium chloride solution. A combined electrode is most frequently used: it should be kept in distilled water.

4.4. Measuring cylinders 50 ml (wine), 100 ml (rectified concentrated must).5. PROCEDURE

5.1. Preparation of sample:

5.1.1. Wines

Elimination of carbon dioxide. Place about 50 ml of wine in a vacuum flask; apply vacuum to the flask with the water pump for one to two minutes, whilst shaking continuously.

5.1.2. Rectified concentrated musts

Introduce 200 g of accurately weighed rectified concentrated must. Make up to the mark with 500 ml water. Homogenize.5.2. Potentiometric titration

5.2.1. Calibration of pH meter

The pH meter is now calibrated for use at 20 °C, according to the manufacturer's instructions, with the pH 7,00 buffer solution at 20 °C.5.2.2. Method of measurement

Into a measuring cylinder (4.4), introduce a volume of the sample, prepared as described in 5.1, equal to 10 ml in the case of wine and 50 ml in the case of rectified concentrated must. Add about 10 ml of distilled water and then add the 0,1 M sodium hydroxide solution (3.2) from the burette until the pH is equal to 7 at 20 °C. The sodium hydroxide must be added slowly and the solution stirred continuously. Let n ml be the volume of 0,1 M NaOH added.5.3. Titration with indicator (bromothymol blue)

5.3.1. Preliminary test: end-point colour determination.

Into a measuring cylinder (4.4) place 25 ml of boiled distilled water, 1 ml of bromothymol blue solution (3.3) and a volume prepared as in (5.1) equal to 10 ml in the case of wine and 50 ml in the case of rectified concentrated must. Add the 0,1 M sodium hydroxide solution (3.2) until the colour changes to blue-green. Then add 5 ml of the pH 7 buffer solution (3.7).5.3.2. Measurement

Into a measuring cylinder (4.4) place 30 ml of boiled distilled water, 1 ml of bromothymol blue solution (3.3) and a volume of the sample, prepared as described in 5.1, equal to 10 ml in the case of wine and 50 ml in the case of rectified concentrated must. Add 0,1 M sodium hydroxide solution (3.2) until the same colour is obtained as in the preliminary test above (5.3.1). Let n ml be the volume of 0,1 M sodium hydroxide added.6. EXPRESSION OF RESULTS

6.1. Method of calculation

6.1.1. Wines

The total acidity expressed in milliequivalents per litre is given by:

A = 10n.It is recorded to one decimal placeThe total acidity expressed in grams of tartaric acid per litre is given by:

A2 = 0,075A

It is recorded to one decimal place.6.1.2. Rectified concentrated musts

- The total acidity expressed in milliequivalents per kilogram of rectified concentrated must is given by a = 5n.
-The total acidity expressed in milliequivalents per kilogram of total sugars is given byP = % concentration (m/m) of total sugars.
It is recorded to one decimal place.6.2. Repeatability (r) for titration with the indicator:

r = 0,9 meq/litrer = 0,07 g tartaric acid/litrefor white, rosé and red wines.6.3. Reproducibility (R) for titration with the indicator (5.3):

For white and rosé wines:R = 3,6 meq/litreR = 0,3 g tartaric acid/litre

For red wines:R = 5,1 meq/litreR = 0,4 g tartaric acid/litre

14. VOLATILE ACIDITY 1. DEFINITION

The volatile acidity is formed from the acids of the acetic series present in wine in the free state and combined as a salt.2. PRINCIPLE OF THE METHOD

Titration of the volatile acids separated from the wine by steam distillation and titration of the distillate.

Carbon dioxide is first removed from the wine.

The acidity of free and combined sulphur dioxide distilled under these conditions should be deducted from the acidity of the distillate.

The acidity of any sorbic acid which may have been added to the wine must also be deducted.

Note: Part of the salicylic acid used in some countries to stabilize the wines before analysis is present in the distillate. This must be determined and deducted from the acidity. The method of determination is given in section 7 of this chapter.3. REAGENTS

3.1. Crystalline tartaric acid ($C_4H_6O_6$).

3.2. 0,1 M sodium hydroxide solution (NaOH).

3.3. 1 % phenolphthalein solution in 96 % vol neutral alcohol.

3.4. Hydrochloric acid (r20 = 1,18 to 1,19 g/ml) diluted 1/4 (v/v).

3.5. 0,005 M iodine (I_2) solution.

3.6. Crystalline potassium iodide (KI).

3.7. 5 g/l starch solution.Mix 5 g of starch with about 500 ml of water. Bring to the boil, stirring continuously and boil for 10 minutes. Add 200 g sodium chloride. When cool, make up to one litre.

3.8. Saturated solution of sodium borate ($Na_2B_4O_7 \cdot 10H_2O$), i.e. about 55 g/l at 20 °C.4. APPARATUS

4.1. Steam distillation apparatus consisting of:1. a steam generator; the steam must be free of carbon dioxide; 2. a flask with steam pipe; 3. a distillation column; 4. a condenser.This equipment must pass the following three tests:(a) Place 20 ml of boiled water in the flask. Collect 250 ml of the distillate and add to it 0,1 ml of 0,1 M sodium hydroxide solution (3.2) and two drops of the phenolphthalein solution (3.3). The pink colouration must be stable for at least 10 seconds (i.e. steam to be free of carbon dioxide).(b) Place 20 ml of a 0,1 M acetic acid solution in the flask. Collect 250 ml of the distillate. Titrate with the 0,1 M sodium hydroxide solution (3.2): the

volume of this used must be at least 19,9 ml (i.e. at least 99,5 % of the acetic acid entrained with the steam).(c) Place 20 ml of 1 M lactic acid solution in the flask. Collect 250 ml of the distillate and titrate the acid with the 0,1 M sodium hydroxide solution (3.2).The volume of sodium hydroxide solution added must be less than or equal to 1,0 ml (i.e. not more than 0,5 % of lactic acid is distilled).Any apparatus or procedure which passes these tests satisfactorily fulfils the requirements of official international apparatus or procedures.

4.2. Water pump.

4.3. Vacuum flask.5. PROCEDURE

5.1. Preparation of sample:elimination of carbon dioxide. Place about 50 ml of wine in a vacuum flask; apply vacuum to the flask with the water pump for one to two minutes, shaking continuously.5.2. Steam distillation

Place 20 ml of wine, freed from carbon dioxide as in 5.1, in the flask. Add about 0,5 g of tartaric acid (3.1). Collect at least 250 ml of the distillate.5.3. Titration

Titrate with the 0,1 M sodium hydroxide solution (3.2) using two drops of phenolphthalein (3.3) as indicator. Let n ml be the volume of sodium hydroxide used.

Add four drops of 1/4 dilute hydrochloric acid (3.4), 2 ml starch solution (3.3) and a few crystals of potassium iodide (3.6). Titrate the free sulphur dioxide with the 0,005 M iodine solution (3.5). Let n" ml be the volume used.

Add the saturated sodium borate solution (3.8) until the pink coloration reappears. Titrate the combined sulphur dioxide with the 0,005 M iodine solution (3.5). Let n" ml be the volume used.6. EXPRESSION OF RESULTS

6.1.Method of calculation

The volatile acidity, expressed in milliequivalents per litre to one decimal place, is given by:A = 5 (n 0,1 n2 0,05 n").

The volatile acidity, expressed in grams of acetic acid per litre to two decimal places, is given by:0,300 (n 0,1 n2 0,05 n").6.2. Repeatability (r)

r= 0,7 meq/litrer = 0,04 g acetic acid/litre.6.3. Reproducibility (R)

R = 1,3 meq/litreR = 0,08 g acetic acid/litre.
6.4. Wine with sorbic acid present

Since 96 % of sorbic acid is steam distilled with a distillate volume of 250 ml, its acidity must be deducted from the volatile acidity, knowing that 100 mg of sorbic acid corresponds to an acidity of 0,89 milliequivalents or 0,053 g of acetic acid and knowing the concentration of sorbic acid in mg/l as determined by other methods.7. DETERMINATION OF SALICYLIC ACID ENTRAINED IN THE DISTILLATE FROM THE VOLATILE ACIDITY

7.1. Principle

After the determination of the volatile acidity and the correction for sulphur dioxide, the presence of salicylic acid is indicated, after acidification, by the violet colouration that appears when an iron (III) salt is added.

The determination of the salicylic acid entrained in the distillate with the volatile acidity is carried out on a second distillate having the same volume as that on which the determination of volatile acidity was carried out. In this distillate, the salicylic acid is determined by a comparative colorimetric method. It is deducted from the acidity of the volatile acidity distillate.7.2. Reagents

7.2.1. Hydrochloric acid (HCl) (r20 = 1,18 to 1,19 g/l).

7.2.2. Sodium thiosulphate, ($Na_2S_2O_3 \cdot 5H_2O$) in a 0,1 M solution.

7.2.3. 10 % (m/v) solution of iron (III) ammonium sulphate ($Fe_2(SO_4)_3 \cdot (NH_4)_2SO_4 \cdot 24H_2O$).

7.2.4. 0,01 M solution of sodium salicylate.
Solution containing 1,60 g/l of sodium salicylate ($NaC_7H_5O_3$).7.3. Procedure

7.3.1. Identification of salicylic acid in the volatile acidity distillate.

Immediately after the determination of the volatile acidity and the correction for free and combined sulphur dioxide, introduce into a conical flask 0,5 ml hydrochloric acid (7.2.1), 3 ml of the 0,1 M sodium thiosulphate solution (7.2.2) and 1 ml of the iron (III) ammonium sulphate solution (7.2.3).

If salicylic acid is present, a violet coloration appears.7.3.2. Determination of salicylic acid

On the above conical flask, indicate the volume of the distillate by a reference mark. Empty and rinse the flask.

Subject a new test sample of 20 ml wine to steam distillation and collect the distillate in the conical flask up to the reference mark. Add 0,3 ml pure hydrochloric acid (7.2.1), and 1 ml of the iron (III) ammonium sulphate solution (7.2.3). The contents of the conical flask turn violet.

Into a conical flask identical to that carrying the reference mark, introduce distilled water up to the same level as that of the distillate. Add 0,3 ml pure hydrochloric acid (7.2.1) and 1 ml of the iron (III) ammonium sulphate solution (7.2.3). From the burette run in the 0,01 M sodium salicylate solution (7.2.4) until the violet coloration obtained has the same intensity as that of the conical flask containing the wine distillate.

Let n" ml be the volume of solution added from the burette.
7.3.3. Correction to the volatile acidity

Subtract the volume 0,1 × n4 ml from the volume n ml of 0,1 M sodium hydroxide solution used to titrate the acidity of the distillate during the determination of volatile acidity.

15. FIXED ACIDITY 1. PRINCIPLE

The fixed acidity is calculated from the difference between total acidity and volatile acidity.2. EXPRESSION OF RESULTS

The fixed acidity is expressed in:- milliequivalents per litre,- grams of tartaric acid per litre.

16. TARTARIC ACID 1. PRINCIPLE OF METHODS

1.1. Reference method

Tartaric acid is precipitated in the form of calcium (±)tartrate and determined gravimetrically. This determination may be completed by a volumetric procedure for comparison. The conditions for precipitation (pH, total volume used, concentrations of precipitating ions) are such that precipitation of the calcium (±)tartrate is complete whereas the calcium D( ) tartrate remains in solution.

When mesotartaric acid has been added to the wine, which causes the precipitation of the calcium (±)tartrate to be incomplete, it must first be hydrolysed.1.2. Usual method

The tartaric acid, separated using an ion exchange column, is determined colorimetrically in the eluate by measurement of the red colour produced on reaction with vanadic acid. The eluate also contains lactic and malic acids which do not interfere.2. REFERENCE METHOD

2.1. Gravimetric method

2.1.1. Reagents

2.1.1.1. Calcium acetate solution containing 10 g of calcium per litre: calcium carbonate ($CaCO_3$)25 gacetic acid glacial (CH_3COOH) (r20 = 1,05 g/ml)40 mlwater to 1 litre

2.1.1.2. Calcium (±)tartrate, crystallized: $CaC_4O_6H_4 \cdot 4H_2O$:Place 20 ml of L(+) tartaric acid solution (5 g/l) into a 400 ml beaker. Add 20 ml of ammonium D( ) tartrate solution (6,126 g/l) and 6 ml of calcium acetate solution containing 10 g of calcium per litre (2.1.1.1).Allow to stand for two hours to precipitate. Collect the precipitate in a sintered glass crucible of porosity No 4, and wash it three times with about 30 ml of distilled water. Dry to constant weight in the oven at 70 °C. Using the quantities of reagent indicated above, about 340 mg of crystallized calcium (±)tartrate is obtained.

Store in a stoppered bottle.

2.1.1.3. Precipitation solution (pH 4,75):- D( ) tartaric acid 122 mg- 25 % (v/v) ammonium hydroxide solution (r20 = 0,97 g/ml) 0,3 ml- calcium acetate solution (10 g calcium/litre) 8,8 ml- water to1 000 ml

Dissolve the D( ) tartaric acid, add the ammonium hydroxide and make up to about 900 ml; add 8,8 ml of calcium acetate solution (2.1.1), make up to a litre and adjust the pH to 4,75 with acetic acid. Since calcium (±)tartrate is slightly soluble in this solution, add 5 mg of calcium (±)tartrate per litre, stir for 12 hours and filter.2.1.2. Procedure

2.1.2.1. Wines with no added mesotartaric acid

Place 500 ml of precipitation solution and 10 ml of wine into a 600 ml beaker. Mix and initiate precipitation by rubbing the sides of the vessel with the tip of a glass rod. Leave to precipitate for 12 hours (overnight).

Filter the liquid and precipitate through a weighed sintered glass crucible of porosity No 4 fitted on a clean vacuum flask. Rinse the vessel in which precipitation took place with the filtrate to ensure that all precipitate is transferred.

Dry to constant weight in an oven at 70 °C. Weigh. Let p be the weight of crystallized calcium (±)tartrate ($CaC_4O_6H_4 \cdot 4H_2O$) obtained.2.1.2.2. Wines to which mesotartaric acid has been added

When analysing wines to which mesotartaric acid has been or is suspected of having been added, proceed by first hydrolysing this acid as follows:

Place 10 ml of wine and 0,4 ml of glacial acetic acid (CH_3COOH, r20 = 1,05 g/ml) into a 50-ml conical flask. Place a reflux condenser on top of the flask and boil for 30 minutes. Allow to cool and then transfer the solution in the conical flask to a 600-ml beaker. Rinse the flask twice using 5 ml of water each time and then continue as described above.

Mesotartaric acid is calculated and included as tartaric acid in the final result.2.1.3. Expression of results

One molecule of calcium (±)tartrate corresponds to half a molecule of L(+) tartaric acid in the wine.

The quantity of tartaric acid per litre of wine, expressed in milliequivalents is equal to 384,5 p.It is quoted to one decimal place.

The quantity of tartaric acid per litre of wine, expressed in grams of tartaric acid is equal to 28,84 p.It is quoted to one decimal place.

The quantity of tartaric acid per litre of wine, expressed in grams of potassium acid tartrate is equal to 36,15 p.

It is quoted to one decimal place.2.2. Comparative volumetric analysis

2.2.1. Reagents

2.2.1.1. Hydrochloric acid (HCl) (1:5 v/v) (r20 = 1,18 to 1,19 g/ml)

2.2.1.2. EDTA solution, 0,05 M:EDTA (ethylenediaminetetraacetic acid disodium salt: $(C_{10}H_{14}N_2O_8Na_2 \cdot 2H_2O)$ 18,61 gdistilled water to1 000 ml

2.2.1.3. Sodium hydroxide solution, 40 % (m/v):sodium hydroxide (NaOH) 40 gdistilled water to 100 ml

2.2.1.4. Complexometric indicator: 1 % (m/m)2-hydroxy-1-(2-hydroxy-4-sulpho-1-naphthylazo)-3-naphthoic acid ($C_{21}H_{14}N_2O_7S \cdot 3H_2O$) 1 ganhydrous sodium sulphate (Na_2SO_4)100 g2.2.2. Procedure

After weighing, replace the sintered glass crucible containing the precipitate of calcium (±)tartrate on the vacuum flask and dissolve the precipitate with 10 ml of dilute hydrochloric acid (2.2.1.1). Wash the sintered glass crucible with 50 ml of distilled water.

Add 5 ml of 40 % sodium hydroxide solution (2.2.1.3) and about 30 mg of indicator (2.2.1.4). Titrate with 0,05 M EDTA (2.2.1.2). Let the number of ml used be n.2.2.3. Expression of results

The quantity of tartaric acid per litre of wine, expressed in milliequivalents is equal to 5 n.It is quoted to one decimal place.

The quantity of tartaric acid per litre of wine, expressed in grams of tartaric acid is equal to 0,375 n.It is quoted to one decimal place.

The quantity of tartaric acid per litre of wine, expressed in grams of potassium acid tartrate is equal to 0,470 n.It is quoted to one decimal place.3. USUAL METHOD

3.1. Reagents

3.1.1. For preliminary treatment of the wine

3.1.1.1. Acetic acid (CH_3COOH; r20 = 1,05 g/ml), diluted 30 % (v/v).

3.1.1.2. A strongly basic ion exchange resin (e.g. anion exchange resin of Merck 20-50 mesh basicity strength III) in acetate form. Prepare a suspension of the ion exchange resin (about

100 g) in 200 ml 30 % acetic acid (3.1.1.1). Leave in contact for at least 24 hours before use. Keep the ion exchange resin in the 30 % acetic acid for later determinations.

3.1.1.3. Acetic acid (CH_3COOH; r20 = 1,05 g/ml), diluted 0,5 % (v/v).

3.1.1.4. Sodium sulphate solution 7,1 g per 100 ml (0,5 M)

Dissolve 71 g of anhydrous sodium sulphate (Na_2SO_4) in distilled water and make up to 1 000 ml with distilled water.3.1.2. For the determination of tartaric acid

3.1.2.1. Sodium acetate solution ($CH_3CO\ Na$) 27 % (m/v):

Dissolve 270 g of anhydrous sodium acetate (CH_3COONa) in distilled water and make up to 1 000 ml.

3.1.2.2. Vanadic reagent:

Dissolve 10 g of ammonium metavanadate (NH_4VO_3) in 150 ml of 1 M sodium hydroxide solution (3.1.2.10). Transfer this solution to a 500 ml volumetric flask and add 200 ml of the 27 % sodium acetate solution (3.1.2.1). Make up to 500 ml with distilled water.

3.1.2.3. 1 M H_2SO_4 solution

3.1.2.4. 0,5 M H_2SO_4 solution

3.1.2.5. 0,05 M H_2SO_4 solution

3.1.2.6. 0,05 M periodic acid solution:

Introduce 10,696 g of sodium periodate, $NaIO_4$, and 50 ml of 0,5 M sulphuric acid (3.1.2.4) into a 1 000 ml volumetric flask and make up to 1 000 ml with distilled water.

3.1.2.7. Glycerol solution, 10 % (m/v):

Place 10 g of glycerol, $C_3H_8O_3$, into a 100 ml volumetric flask and make up to 100 ml with distilled water.

3.1.2.8. Sodium sulphate solution, 7,1 g per 100 ml (see 3.1.1.4).

3.1.2.9. Tartaric acid solution, 1 g/l:

Introduce 0,5 g of tartaric acid and 6,66 ml of 1 M sodium hydroxide solution (3.1.2.10) into a 500 ml volumetric flask and make up to 500 ml with the 7,1 % sodium sulphate solution (3.1.1.4).

3.1.2.10. Sodium hydroxide solution, NaOH, 1 M.3.2.Apparatus

3.2.1. Glass column of 10 to 11 mm internal diameter and approximately 300 mm long fitted with a drain tap.

3.2.2. Spectrophotometer enabling absorbence measurements to be made at a wavelength of 490 nm, having cells with a 10 mm optical path.3.3. Procedure

3.3.1. Preparation of the ion exchange column

Place a glass wool plug in the glass column above the drain tap (3.2.1). Soak this plug with distilled water. Pour into the column 10 ml of the suspension of ion exchange resin in ace tate form (3.1.1.2). Allow to settle. Place a plug of glass wool on top of the resin (to prevent it being disturbed during subsequent washings).

The ion exchange resin must only be used for one operation.3.3.2. Separation of organic acids

With the tap open, allow the acetic acid solution to flow down the column to within approximately 2 to 3 mm of the upper glass wool plug.

Add 10 ml of the 0,5 % acetic acid solution (3.1.1.3) and allow the liquid to drain again down to the same level as the previous operation. Repeat this washing operation four more times.

After the last wash, close the tap, and pour 10 ml of the wine or must on to the ion exchange resin. Allow it to flow a drop at a time (average rate one drop per second) and stop the flow just above the ion exchange resin. Once more add 10 ml of the 0,5 % acetic acid solution (3.1.1.3) to the column, allow to drain at the same rate as previously and subsequently wash seven times in the same way using 10 ml of water each time. During the last washing, close the tap as soon as the liquid level is just above the upper glass wool plug.

Elute the acids absorbed on the ion exchange resin with a 7,1 % sodium sulphate solution (3.1.1.4). Collect the eluate in a 100 ml graduated flask up to the calibration mark.3.3.3. Determination of tartaric acid

3.3.3.1. Wines with no added mesotartaric acid

Place 20 ml of eluate in two conical flasks a and b.

Use flask a for the determination and flask b, in which the tartaric acid has been destroyed by periodic acid, for the blank experiment.

Introduce into flaska:- 2 ml of M H2SO4 (3.1.2.3),- 5 ml of 0,05 M H2SO4 (3.1.2.5),- 1 ml of 10 % glycerol (3.1.2.7).

Introduce into flask b:- 2 ml of M H2SO4 (3.1.2.3),- 5 ml of 0,05 M periodic acid solution (3.1.2.6).

Wait 15 minutes; add 1 ml of 10 % glycerol (3.1.2.7) to destroy the excess periodic acid.

Wait two minutes.

Then, while stirring, pipette 5 ml of the vanadic reagent (3.1.2.2) first into flask b and then immediately afterwards into flask a. Immediately start a timer and pour the contents of flasks a and b into the spectrophotometer cells. After 90 seconds, record the absorbence at 490 nm of the liquid from flask a (determination) with respect to liquid b (blank).

Solutions containing high levels of tartaric acid that give absorbencies that are above the top standard should be diluted with 7,1 % sodium sulphate and then measured as before.
3.3.3.2. Wines to which mesotartaric acid has been added

When analysing wines to which mesotartaric acid has been or is suspected of having been added, proceed by first hydrolysing this acid as described for the reference method.

After cooling, the contents of the conical flask are poured into the top of the ion exchange column, followed by rinsing water (5 ml, twice). Continue as indicated above.

The mesotartaric acid is included as tartaric acid in the final result.3.3.4.Plotting the calibration curve

Pipette 10, 20, 30, 40 and 50 ml of the 1 g/l tartaric acid solution (3.1.2.9) into graduated 100 ml flasks and make up to 100 ml with the 7,1 % sodium sulphate solution (3.1.1.4). The concentrations of these solutions correspond to wine eluates containing 1, 2, 3, 4 and 5 g/l of tartaric acid.

Introduce into two conical flasks a and b 20 ml of these solutions and continue as described above for the wine eluate.

A graph of the absorbences of these solutions as a function of their tartaric acid concentration is a straight line curving slightly inwards towards the origin. If necessary, draw this part of the curve more carefully using determinations on solutions of accurately known concentrations below 1,0 g/l.3.3.5. Expression of results

The absorbence measured for the eluate is found on the calibration curve and gives the tartaric acid concentration in the wine in grams per litre.The result is expressed to one decimal place.

17. CITRIC ACID 1. PRINCIPLE OF THE METHOD

Citric acid is converted into oxaloacetate and acetate in a reaction catalysed by citrate-lyase (CL):citrate oxaloacetate + acetate

In the presence of malate dehydrogenase (MDH) and lactate dehydrogenase (LDH), the oxaloacetate and its decarboxylation derivative, pyruvate, are reduced to L-malate and L-lactate by reduced nicotinamide adenine dinucleotide (NADH):oxaloacetate + NADH + H+ L-malate + NAD+pyruvate + NADH + H+ L-lactate + NAD+

The amount of NADH oxidized to NAD+ in these reactions is proportional to the amount of citrate present. The oxidation of NADH is measured by the resultant decrease in absorbence at a wavelength of 340 nm.2. REAGENTS

2.1. Buffer solution pH 7,8.
(0,51 M glycylglycine; pH 7,8; Zn2+ (0,6 × 10 3M):dissolve 7,13 g of glycylglycine in approximately 70 ml of doubly distilled water.

Adjust the pH to 7,8 with approximately 13 ml of 5 M sodium hydroxide solution, add 10 ml of zinc chloride (ZnCl2 80 mg in 100 ml H2O) solution and make up to 100 ml with doubly distilled water.

2.2. Reduced nicotinamide adenine dinucleotide (NADH) solution (approximately 6 × 10 3 M): dissolve 30 mg NADH and 60 mg NaHCO3 in 6 ml of doubly distilled water.

2.3. Malate dehydrogenase/lactate dehydrogenase solution (MDH/LDH, 0,5 mg MDH/ml, 2,5 mg LDH/ml): mix together 0,1 ml MDH (5 mg MDH/ml), 0,4 ml ammonium sulphate solution (3,2 M and 0,5 ml LDH (5 mg/ml). This suspension remains stable for at least a year at 4 °C.

2.4. Citrate-lyase (CL, 5 mg protein/ml): dissolve 168 mg lyophilisate in 1 ml ice-cold water. This solution remains stable for at least a week at 4 °C and for at least four weeks if frozen.

It is recommended that, prior to the determination, the enzyme activity should be checked.

2.5. Polyvinylpolypyrrolidone (PVPP)

Note: All the reagents above are available commercially.3. APPARATUS

3.1. A spectrophotometer permitting measurement to be made at 340 nm, the wavelength at which absorption by NADH is at a maximum.

Failing that, a spectrophotometer, with a discontinuous spectrum source permitting measurements to be made at 334 nm or 365 nm, may be used.

Since absolute absorbence measurements are involved (i.e. calibration curves are not used but standardization is made by consideration of the extinction coefficient of NADH), the wavelength

scales and spectral absorbence of the apparatus must be checked.

3.2. Glass cells with optical path lengths of 1 cm or single-use cells.

3.3. Micropipettes for pipetting volumes in the range 0,02 to 2 ml.
4. PREPARATION OF THE SAMPLE

Citrate determination is normally carried out directly on the wine, without preliminary removal of pigmentation (colouration) and without dilution provided that the citric acid content is less than 400 mg/l. If this is not so, dilute the wine until the citrate concentration lies between 20 and 400 mg/l (i.e. between 5 and 80 µg of citrate in the test sample).

With red wines that are rich in phenolic compounds, preliminary treatment with PVPP is recommended:

Form a suspension of about 0,2 g of PVPP in water and allow to stand for 15 minutes. Filter using a fluted filter.

Place 10 ml of wine in a 50 ml conical flask, add the moist PVPP removed from the filter with a spatula. Shake for two to three minutes. Filter.5. PROCEDURE

With the spectrophotometer adjusted to a wavelength of 340 nm, determine the absorbence using the 1 cm cells, using air as the zero absorbence (reference) standard (no cell in the optical path). Place the following in the 1 cm cells:

Reference
cell
(ml) Sample
cell
(ml)Solution 2.1 1,00 1,00Solution 2.2 0,10 0,10Sample to be measured - 0,20Doubly distilled water 2,00 1,80Solution 2.3 0,02 0,02

Mix, and after about five minutes read the absorbence of the solutions in the reference and sample cells (A1).

Add:Solution 2.4 0,02 ml 0,02 ml

Mix; wait until the reaction is completed (about five minutes) and read the absorbences of the solutions in the reference and sample cells (A2).

Calculate the absorbence difference (A2 - A1) for the reference and sample cells, DAR and DAS.Finally, calculate the difference between those differences:DA = DAs DAR

Note: The time needed for the completion of enzyme activity can vary from one batch to another. The above value is given only for guidance and it is recommended that it be determined for each batch.6. EXPRESSION OF RESULTS

Citric acid concentration is given in milligrams per litre to the nearest whole number.6.1. Method of calculation

The general formula for calculating the concentration in mg/l is:
where V = volume of test solution in ml (here 3,14 ml)v = volume of the sample in ml (here 0,2 ml)M = molecular mass of the substance to be determined
(here, for anhydrous citric acid, M = 192,1)d = optical path in the cell in cm (here, 1 cm)e = absorption coefficient of NADH, (at 340 nm, e = 6,3 mmol 1 × l × cm 1),

so thatC = 479 × DA

If the sample was diluted during its preparation, multiply the result by the dilution factor.

Note:At 334 nm: C = 488 × DA (= 6,2 m mol 1 × l × cm 1).At 365 nm: C = 887 × DA (= 3,4 m mol 1 × l × cm 1).6.2. Repeatability (r)

Citric acid concentration less than 400 mg/l: r = 14 mg/l.Citric acid concentration greater than 400 mg/l: r = 28 mg/l.6.3. Reproducibility (R)

Citric acid concentration less than 400 mg/l: R = 39 mg/l.Citric acid concentration greater than 400 mg/l: R = 65 mg/l.

18. LACTIC ACID 1. PRINCIPLE OF THE METHOD

Total lactic acid (L-lactate and D-lactate) is oxidized by nicotinamide adenine dinucleotide (NAD) to pyruvate in a reaction catalysed by L-lactate dehydrogenase (L-LDH) and D-lactate dehydrogenase (D-LDH).

The equilibrium of the reaction normally lies more strongly in favour of the lactate. Removal of the pyruvate from the reaction mixture displaces the equilibrium towards the formation of pyruvate.

In the presence of L-glutamate, the pyruvate is transformed into L-alanine in a reaction catalysed by glutamate pyruvate transaminase (GPT):(1) L-lactate + NAD+ pyruvate + NADH + H+(2) D-lactate + NAD+ pyruvate + NADH + H+(3) Pyruvate + L-glutamate L-alanine + a-ketoglutarate

The amount of NADH formed, measured by the increase in absorbence at the wavelength of 340 nm, is proportional to the quantity of lactate originally present.

Note:L-lactic acid may be determined independently by using reactions (1) and (3), while D-lactic acid may be similarly determined by using reactions (2) and (3).1.2. Usual method

The lactic acid, separated by passage through an ion exchange resin column, is oxidized to ethanal and determined by colorimetry after reacting with sodium nitroprusside and piperidine.

2. REFERENCE METHOD

2.1. Reagents

2.1.1. Buffer solution, pH 10 (glycylglycine 0,6 mol/l; L-glutamate 0,1 mol/l):

dissolve 4,75 g of glycylglycine and 0,88 g of L-glutamic acid in approximately 50 ml of doubly distilled water; adjust the pH to 10 with a few millilitres of 10 M sodium hydroxide and make up to 60 ml with doubly distilled water.

This solution will remain stable for at least 12 weeks at 4 °C.

2.1.2. Nicotinamide adenine dinucleotide (NAD) solution, approximately 40 × 10 3 M: dissolve 900 mg of NAD in 30 ml of doubly distilled water. This solution will remain stable for at least four weeks at 4 °C.

2.1.3. Glutamate pyruvate transaminase (GPT) suspension, 20 mg/ml. The suspension remains stable for at least a year at 4 °C.

2.1.4. L-lactate dehydrogenase (L-LDH) suspension, 5 mg/ml. This suspension remains stable for at least a year at 4 °C.

2.1.5. D-lactate dehydrogenase (D-LDH) suspension, 5 mg/ml. This suspension remains stable for at least a year at 4 °C.

It is recommended that, prior to the determination, the enzyme activity should be checked.

Note: All the reagents are available commercially.2.2. Apparatus

2.2.1. A spectrophotometer permitting measurements to be made at 340 nm, the wavelength at which absorption by NADH is at a maximum.

Failing that, a spectrophotometer with a discontinuous spectrum source permitting measurements to be made at 334 or 365 nm may be used.

Since absolute absorbence measurements are involved (i.e. calibration curves are not used, but standardization is made by consideration of the extinction coefficient of NADH), the wavelength scales and spectral absorbence of the apparatus must be checked.

2.2.2. Glass cells with optical path lengths of 1 cm or single-use cells.

2.2.3. Micropipettes for pipetting sample volumes in the range 0,02 to 2 ml.2.3. Preparation of the sample

Preliminary note: No part of the glassware that comes into contact with the reaction mixture should be touched with the fingers, since this could introduce L-lactic acid and thus give erroneous results.

Lactate determination is normally carried out directly on the wine, without prior removal of pigmentation (colouration) and without dilution provided that the lactic acid concentration is less than 100 mg/l. If, however, the lactic acid concentration lies between:- 100 mg/l and 1 g/l, dilute 1/10 with doubly distilled water,- 1 g/l and 2,5 g/l, dilute 1/25 with doubly distilled water,- 2,5 g/l and 5 g/l, dilute 1/50 with doubly distilled water.2.4. Procedure

2.4.1. Determination of total lactic acid

The buffer solution must be at a temperature between 20 and 25 °C before proceeding to the measurement.

With the spectrophotometer adjusted to a wavelength of 340 nm, determine the absorbence using the cells having optical paths of 1 cm, with air as the zero absorbence (reference) standard (no cell in the optical path) or with water as the standard.

Place the following in the cells having 1 cm optical paths:

Reference
cell
(ml) Sample
cell
(ml)Solution 2.1.1 1,00 1,00Solution 2.1.2 0,20 0,20Doubly distilled water 1,00 0,80Suspension 2.1.3 0,02 0,02Sample to be measured - 0,20

Mix using a glass stirrer or a rod of synthetic material with a flattened end; after about five minutes, measure the absorbences of the solutions in the reference and sample cells (A1).

Add 0,02 ml of solution 2.1.4 and 0,05 ml of solution 2.1.5, homogenize, wait for the reaction to be completed (about 30 minutes) and measure the absorbences of the solutions in the reference and sample cells (A2).

Calculate the differences (A2 A1) in the absorbences of the solutions in the reference and sample cells, DAR and DAS.

Finally, calculate the difference between those differences:

DA = DAS DAR2.4.2. Determination of L-lactic acid and D-lactic acid

Determinations of the L-lactic acid or D-lactic acid can be carried out independently by applying the procedure for total lactic acid up to the determination of A1 and then continuing as follows:

Add 0,02 ml of solution 2.1.4, homogenize, wait until the reaction is complete (about 20 minutes) and measure the absorbences of the solutions in the reference and sample cells (A2).

Add 0,05 ml of solution 2.1.5, homogenize, wait until the reaction is complete (about 30 minutes) and measure the absorbences of the solutions in the reference and sample cells (A3).

Calculate the differences (A2 A1) for L-lactic acid and (A3 A2) for D-lactic acid between the absorbences of the solutions in the reference and sample cells, DAR and DAS.

Finally, calculate the difference between those differences:

DA = DAS DAR

Note:The time needed for the completion of enzyme activity can vary from one batch to another. The above value is given only for guidance and it is recommended that it be determined for each batch. When determining the L-lactic acid alone, the incubation time may be reduced to 10 minutes.2.5. Expression of results

Lactic acid concentration is given in grams per litre to one decimal place.

2.5.1. Method of calculation

The general formula for calculating the concentration in g/l is:whereV = volume of test solution in ml (V = 2,24 ml for L-lactic acid, V = 2,29 ml for D-lactic acid and total lactic acid)v = volume of the sample in ml (here 0,2 ml)M = molecular mass of the substance to be determined (here, for DL-lactic acid,
M= 90,08)d = optical path in the cell in cm (here, 1 cm)e = absorption coefficient of NADH, (at 340 nm, e = 6,3 mmol 1 × l × cm 1).2.5.1.1. Total lactic acid and D-lactic acid

C = 0,164 × DA

If the sample was diluted during its preparation, multiply the result by the dilution factor.

Note:Measurement at 334 nm: C = 0,167 × DA, (e = 6,2 m mol 1 × l × cm 1).Measurement at 365 nm: C = 0,303 × DA, (e = 3,4 m mol 1 × l × cm 1).
2.5.1.2. L-lactic acid

C = 0,160 × DA

If the sample was diluted during its preparation, multiply the result by the dilution factor.

Note:Measurement at 334 nm: C = 0,163 DA, (e = 6,2 m mol 1 × l × cm 1).Measurement at 365 nm: C = 0,297 DA, (e= 3,4 m mol 1 × l × cm 1).2.5.2. Repeatability (r)

r = 0,02 + 0,07xi g/l

xi is the lactic acid concentration in the sample in g/l.2.5.3. Reproducibility (R)

R = 0,05 + 0,125xi g/l

xi is the lactic acid concentration in the sample in g/l.3. USUAL METHOD

3.1.1. For preliminary treatment of the wine

Refer to the chapter 'Tartaric acid', usual method, section 3.1.1.3.1.2. For the determination of lactic acid

3.1.2.1. 0,1 M solution of cerium (IV) sulphate in 0,35 M sulphuric acid:

Keeping the solution cool, dissolve 40,431 g of cerium (IV) sulphate, $Ce(SO4)2 \cdot 4H2O$, in 350 ml of an accurately measured 1 M solution of sulphuric acid (3.1.2.4). Make up to 1 000 ml with distilled water.

3.1.2.2. 2,5 M solution of sodium hydroxide (NaOH).

3.1.2.3. Sodium acetate solution, 270 g per litre (prepared from dry sodium acetate, CH3COONa).

3.1.2.4. 1 M sulphuric acid, H_2SO_4.

3.1.2.5. 2 % (m/v) sodium nitroprusside solution ($Na_2FeNO(CN)_5 \cdot 2H_2O$)

Keep in the dark in a well stoppered bottle.
Do not keep the solution longer than eight hours.

3.1.2.6. 10 % (v/v) solution of piperidine ($C_5H_{11}N$).

3.1.2.7. 1 M solution of lactic acid:

100 ml of lactic acid ($C_3H_6O_3$) are diluted in 400 ml of water. This solution is heated in an evaporating dish over a boiling water bath for four hours, topping up the volume occasionally with distilled water. After cooling, make up to a litre. Titrate the lactic acid in 10 ml of this solution with 1 M sodium hydroxide solution (3.1.2.8). Adjust the solution to 1 M lactic acid (90 g).

3.1.2.8. 1 M sodium hydroxide solution (NaOH).
3.2. Apparatus

3.2.1. Glass column of 10 to 11 mm internal diameter and approximately 300 mm long fitted with a drain tap to regulate the flow.

3.2.2. Constant temperature water bath at 65 °C.

3.2.3. Spectrophotometer enabling absorbence measurements to be made at a wavelength of 570 nm having cells with a 1 cm optical path.3.3. Procedure

3.3.1. Preparation of the ion exchange column

See the chapter 'Tartaric acid', usual method, section 3.3.1.3.3.2. Separation of organic acids

See the chapter 'Tartaric acid', usual method, section 3.3.2.3.3.3. Determination of lactic acid

Place 10 ml of the eluate in a 50 ml glass test tube fitted with a ground glass stopper, and add 10 ml of cerium sulphate solution (3.1.2.1). Stir; place the tube in a constant temperature water bath at 65 °C for exactly 10 minutes. At the time when it is immersed in the bath, remove the glass stopper for a few seconds to compensate for the rise in pressure due to the heating, then insert the stopper so as to seal the tube tightly and avoid losing any of the ethanal (acetaldehyde) formed. Remove the tube from the bath, and cool it under running water to bring it to a temperature of around 20 °C. Add 5 ml of the 2,5 M sodium hydroxide solution (3.1.2.2), mix and filter.

Take 15 ml of the filtrate and pour it into a 50 ml stoppered flask already containing a homogeneous mixture of 5 ml of 27 % sodium acetate solution (3.1.2.3) and 2 ml of 1 M sulphuric acid (3.1.2.4). Add 5 ml of sodium nitroprusside solution (3.1.2.5), mix, then add 5 ml of the piperidine solution (3.1.2.6), mix rapidly and introduce this solution immediately into the spectrophotometer cell. The colouration produced varies from green to violet and is measured at 570 nm with respect to air (no cell in the optical path): it increases and then rapidly decreases.

Follow the corresponding variation in absorbence and choose the maximum value as the definitive value.

If the eluate is too rich in lactic acid and the absorbence too high, dilute the eluate with the 7,1 % sodium sulphate solution (3.1.1) and proceed with the measurement on the diluted solution.3.3.4. Plotting the calibration curve

Pipette 10 ml of the 1 M lactic acid solution (3.1.2.7) and 10 ml of the titrated 1 M sodium hydroxide solution (3.1.2.8) into a 1 000-ml graduated flask and make up to the mark with 7,1 % sodium sulphate solution. Take 5, 10, 15, 20 and 25 ml of this solution and introduce them into 100 ml graduated flasks. Make up to the reference mark with 7,1 % (3.1.1) sodium sulphate solution. Take 10 ml of each of the resultant solutions and determine their absorbences according to the procedure described in 3.3.3 for the eluate.

The concentrations of these solutions correspond to wine eluates containing 0,45, 0,9, 1,35, 1,80 and 2,25 g/l of lactic acid.

A graph of the absorbences of these solutions as a function of their lactic acid concentration is a straight line.
3.4. Expression of results

The absorbence measured for the eluate is found on the calibration curve and gives the lactic acid concentration in the wine in grams per litre.The result is expressed to one decimal place.

Note:Wines containing more than 250 mg/l of sulphur dioxide may have an ethanalsulphonic acid content which is included with that of the lactic acid. In this case, the result must be corrected by an amount given by the following additional operation: 15 ml of the eluate is mixed in a stoppered test tube with 5 ml of 27 % sodium acetate (3.1.2.3) and 2 ml of 0,775 H_2SO_4 (775 ml of 1 M H_2SO_4 is made up to 100 ml with distilled water). Then, as in the determination of lactic acid, add 5 ml of 2 % sodium nitroprusside (3.1.2.5) and 5 ml of 10 % (3.1.1.6) piperidine. After mixing, measure the absorbence under the same conditions as those described for the determination of lactic acid. Locate this absorbence on the calibration curve to obtain the apparent valueB of the lactic acid in grams per litre due to the ethanalsulphonic acid. If L2 is the apparent concentration of the lactic acid in the wine in grams per litre, the real concentration L of lactic acid is given by:

$L = L2\ B \times 0,4$ (g/l)

19. L-MALIC ACID 1. PRINCIPLE OF THE METHOD

L-malic acid (L-malate) is oxidized by nicotinamide adenine dinucleotide (NAD) to oxaloacetate in a reaction catalysed by L-malate dehydrogenase (L-MDH).

The equilibrium of the reaction normally lies more strongly in favour of the malate. Removal of the oxaloacetate from the reaction mixture displaces the equilibrium towards the formation of oxaloacetate. In the presence of L-glutamate, the oxaloacetate is transformed into L-aspartate in a reaction catalysed by glutamate oxaloacetate transaminase (GOT):

(1) L-malate + NAD^+ oxaloacetate + NADH + H^+

(2) Oxaloacetate + L-glutamate L-aspartate + a-ketoglutarate

The amount of NADH formed, measured by the increase in absorbence at the wavelength of 340 nm, is proportional to the quantity of L-malate originally present.2. REAGENTS

2.1. Buffer solution, pH 10
(glycylglycine 0,6 M; L-glutamate 0,1 M):

dissolve 4,75 g of glycylglycine and 0,88 g of L-glutamic acid in approximately 50 ml of doubly distilled water; adjust the pH to 10 with about 4,6 ml of 10 M sodium hydroxide and make up to 60 ml with doubly distilled water.This solution will remain stable for at least 12 weeks at 4 °C.

2.2. Nicotinamide adenine dinucleotide (NAD) solution, approximately 47 × 10 3 M:dissolve 420 mg of NAD in 12 ml of doubly distilled water. This solution will remain stable for at least four weeks at 4 °C.

2.3.Glutamate oxaloacetate transaminase (GOT) suspension, 2 mg/ml. The suspension remains stable for at least a year at 4 °C.

2.4.L-malate dehydrogenase (L-MDH) solution, 5 mg/ml. This solution remains stable for at least a year at 4 °C.

Note: All the reagents above are available commercially.3. APPARATUS

3.1. A spectrophotometer permitting measurement to be made at 340 nm, the wavelength at which absorption by NADH is at a maximum.Failing that, a spectrophotometer, with a discontinuous spectrum source permitting measurements to be made at 334 or 365 nm, may be used.

Since absolute measurements of absorbence are involved (i.e. calibration curves are not used, but standardization is made by consideration of the extinction coefficient of NADH), the wavelength scales and spectral absorbence of the apparatus must be checked.

3.2. Glass cells with optical path lengths of 1 cm or single-use cells.

3.3. Micropipettes for pipetting sample volumes in the range 0,01 to 2 ml.4. PREPARATION OF THE SAMPLE

L-malate determination is normally carried out directly on the wine, without prior removal of pigmentation (colouration) and without dilution provided that the L-malic acid concen-

tration is less than 350 mg/l (measured at 365 mg/l). If this is not so, dilute the wine with doubly distilled water until the L-malate concentration lies between 30 and 350 mg/l (i.e. amount of L-malate in the test sample lies between 3 and 35 μg).

If the malate concentration in the wine is less than 30 mg/l, the volume of the test sample may be increased up to 1 ml. In this case, the volume of water to be added is reduced in such a way that the total volumes in the two cells are equal.5. PROCEDURE

With the spectrophotometer adjusted to a wavelength of 340 nm, determine the absorbence using the cells having optical paths of 1 cm, with air as the zero absorbence (reference) standard (no cell in the optical path) or with water as the standard.

Place the following in the cells having 1 cm optical paths:

Reference
cell
(ml) Sample
cell
(ml)Solution 2.1 1,00 1,00Solution 2.2 0,20 0,20Doubly distilled water 1,00 0,90Suspension 2.3 0,01 0,01Sample to be measured - 0,10

Mix; after about three minutes, measure the absorbences of the solutions in the reference and sample cells (A1).

Add:Solution 2.4 0,01 ml 0,01 ml

Mix; wait for the reaction to be completed (about 5 to 10 minutes) and measure the absorbences of the solutions in the reference and sample cells (A2).

Calculate the differences (A2 A1) in the absorbences of the solutions in the reference and sample cells, DARand DAS.

Finally, calculate the difference between those differences: DA = DAS DAR

Note: The time needed for the completion of enzyme activity can vary from one batch to another. The above value is given only for guidance and it is recommended that it be determined for each batch.6. EXPRESSION OF RESULTS

L-malic acid concentration is given in grams per litre to one decimal place.6.1. Method of calculation

The general formula for calculating the concentration in g/l is:whereV = volume of test solution in ml (here 2,22 ml)v = volume of the sample in ml (here 0,1 ml)M = molecular mass of the substance to be determined (here, for L-malic acid, M=134,09)d = optical path in the cell in cm (here, 1 cm)
e = absorption coefficient of NADH, (at 340 nm,e = 6,3 m mol 1 × l × cm 1),

so that for L-malate:C = 0,473 × DA g/l

If the sample was diluted during its preparation, multiply the result by the dilution factor.

Note: Measurement at 334 nm: C = 0,482 × DA
Measurement at 365 nm: C= 0,876 × DA6.2. Repeatability (r)

r = 0,03 + 0,034xi
xiis the malic acid concentration in the sample in g/l.6.3. Reproducibility (R)

R = 0,05 + 0,071xi
xi is the malic acid concentration in the sample in g/l.

20. D-MALIC ACID (enzymatic method) Test combination for approximately 30 determinations1. PRINCIPLE

In the presence of D-malate dehydrogenase decarboxyl (D-MDH), D-malic acid (D-malate) is oxidized by nicotinamide adenine dinucleotide (NAD) to oxaloacetate. The oxaloacetate formed in this reaction is split into pyruvate and carbonic acid.

D-malate + NAD + D-MDH + pyruvate + CO2 + NADH + H+

The quantity of NADH formed is proportional to the concentration of D-malic acid and is measured at a wavelength of 334, 340 or 365 nm.2. THE TEST COMBINATION CONTAINS:

(a) Bottle 1 with ca. 30 ml of solution consisting of Hepes-buffer 4-(2-hydroxyethyl)-1-piperzeinethan-sulphonic acid), pH=9,0 and stabilizers; (b) Bottle 2 with ca. 210 mg of NAD-lyophilisate; (c) Three bottles with D-MDH-lyophilisate,ca. 8 U each.2.1. Preparation of solutions

2.1.1. Use content of bottle 1 undiluted. Before using bring solution to 20 to 25 °C.

2.1.2. Dissolve content of bottle 2 in 4 ml redistilled water. Before using bring solution to 20 to 25 °C.

2.1.3. Dissolve content of one of bottles 3 in 0,6 ml redistilled water. Before using bring solution to 20 to 25 °C.2.2. Stability of solutions

The content of bottle 2.1.1 is stable for one year if stored at +4 °C.Solution 2.1.2 is stable for three weeks if stored at +4 °C and for two months if stored at 20 °C.Solution 2.1.3 is stable for five days if stored at +4 °C.3. APPARATUS

3.1. A spectrophotometer enabling measurement at 340 nm, the wavelength at which absorption by NADH and NADPH is at a maximum.If the above is not available, a spectrophotometer with a discontinuous spectrum source enabling measurements to be made at 334 or 365 nm may be used.

3.2. Glass cuvettes 1 cm (if preferred disposable cuvettes can be used).

3.3. Micropipettes: suitable for pipetting volumes of 0,01 and 2 ml.4. PREPARATION OF THE SAMPLE

The amount of D-malic acid in the cuvette should be between 2 mg and 50 g. The sample solution therefore must be diluted to yield a D-malic acid concentration between 0,02 and 0,5 g/l or 0,02 and 0,3 g/l, respectively.

If the absorbence differenceD A is < 0,100, the sample volume can be increased up to 1,80 ml. The volume of water to be added must then be reduced so as to obtain an identical final volume in both the cuvettes (sample and blank).
5. PROCEDURE:

Temperature: 20 to 25 °C.Final volume: 2,95 ml.

Read against air (without a cuvette in the light path), against water or against blank.

Pipette into cuvettesblanksampleSolution 2.1.11,00 ml1,00 mlSolution 2.1.20,10 ml0,10 mlRedistilled water1,80 ml1,70 mlSample solution 0,10 mlMix, read absorbencies of the solutions (A1) after approximately six minutes. Start reaction by addition ofSolution 2.1.30,05 ml0,05 mlMix; on completion of the reaction (approximately 20 minutes) read absorbences of the solutions (A2).

Calculate the absorbence differences (A2 A1) for both blank and sample. Subtract the absorbence difference of the blank (D AB) from the absorbence difference of the sample (D AS):D A = D AS D AB6. EXPRESSION OF RESULTS:

The concentration of D-malic acid is given in g/l to one decimal place.6.1. Calculation

According to the general formula for calculating the concentrations, the equation is:where V = final volume (ml)v = sample volume (ml)M = molecular weight of the substance to be assayedd = light path (cm)e = absorption coefficient of NADH atHg 340 nm = 6,3 (mmol 1 × l × cm 1)Hg 365 nm = 3,4 (mmol 1 × l × cm 1)Hg 334 nm = 6,18 (mmol 1 × l × cm 1)

It follows from D-malic acid:If a dilution has been made when preparing the sample, the result must be multiplied by the dilution factor F.
6.2. Reproducibility (r)

r = 0,05 Xixi = concentration of D-malic acid in g/l.6.3. Reproducibility (R)

R = 0,1 Xi
Xi = concentration of D-malic acid in g/l.

21. TOTAL MALIC ACID 1. PRINCIPLE

Malic acid, separated by means of an anion exchange column, is determined colorimetrically in the eluent by measuring the yellow coloration it forms with chromotropic acid in the presence of concentrated sulphuric acid. A correction for interfering substances is made by subtracting the absorbence, obtained using 86 % sulphuric and chromotropic acid respectively (malic acid does not react at these acid concentrations), from the absorbence obtained from using 96 % strength acids.2. APPARATUS

2.1. Glass column approximately 250 mm in length and 35 mm internal diameter, fitted with drain tap.

2.2. Glass column approximately 300 mm in length and 10 to 11 mm internal diameter, fitted with drain tap.

2.3. Thermostatically controlled water bath at 100 °C.

2.4. Spectrophotometer set to measure absorbence at 420 nm using 10 mm cells.3. REAGENTS

3.1. A strongly basic ion exchange resin (e.g. Merck III).

3.2. Sodium hydroxide 5 % (m/v).

3.3. Acetic acid 30 % (m/v).

3.4. Acetic acid 0,5 % (m/v).

3.5. Sodium sulphate solution 10 % (m/v).

3.6. Concentrated sulphuric acid 95 to 97 % (m/m).

3.7. Sulphuric acid 86 % (m/m).

3.8. Chromotropic acid 5 % (m/v)

Prepare fresh solution before each determination by dissolving 500 mg sodium chromotropate, (C10H6Na2O8S2.2H20) in 10 ml distilled water.

3.9. DL-Malic acid solution 0,5 g/l.

Dissolve 250 g malic acid (C4H6O5) in sodium sulphate solution (10 %), make up to 500 ml with sodium sulphate solution (10 %) (3.5).4. PROCEDURE

4.1. Preparation of ion exchange resin

Place a plug of cotton wool impregnated with distilled water at the bottom of the column (35×250 mm) above the tap. Pour a suspension of the anion exchange resin into the glass column. The level of the liquid should be 50 mm above the top of the resin. Rinse with 1 000 ml of distilled water. Wash the column with sodium hydroxide solution (5 %), allow to drain to within 2 to 3 mm of the top of the resin and repeat with two further washings of sodium hydroxide 5 % and leave for one hour. Wash the column with 1 000 ml of distilled water. Refill the column with acetic (30 %) acid, allow to drain to within 2 to 3 mm from the top of the column and repeat with two further washings of acetic acid (30 %). Leave for at least 24 hours before use. Keep the ion exchange resin in acetic acid (30 %) for the subsequent analyses.4.2. Preparation of ion exchange column

Place a plug of cotton wool at the bottom of the column (11 × 300 mm) above the tap. Pour in the suspension of ion exchange resin (prepared in 4.1) to a height of 10 cm. Open the tap and allow the acetic acid solution (30 %) to drain to within 2 to 3 mm of the top of the resin. Wash with a 50 ml portion of acetic acid (0,5 %).4.3. Separation of DL-malic acid

Pour onto the column (prepared in 4.2) 10 ml of wine or must. Allow to drain one drop at a time (average rate of one drop per second) and stop the flow 2 to 3 mm from the top of the resin. Wash the column with 50 ml acetic acid (0,5 %) then with 50 ml of distilled water and allow to drain at the same rate as previously, stopping the flow 2 to 3 mm from the top of the resin.

Elute the acids absorbed on the exchange resin with a 10 % sodium sulphate solution (3.5). Collect the eluate in a 100 ml volumetric flask.

The column can be regenerated using the procedure desribed in (4.1).4.4. Determination of malic acid

Label two wide necked 30-ml tubes (fitted with ground glass stoppers) A and B. Into each tube add 1,0 ml of the eluent (4.3) and 1 ml of chromotropic acid (5 %). Add 10 ml sulphuric acid (86 %) (reference) to tube A and 10 ml (96 %) sulphuric acid to tube B (sample). Stopper and shake to homogenize, taking care not to wet the glass stopper. Immerse the tubes in a boiling water bath for exactly 10 minutes. Cool the tubes in the dark at 20 °C for exactly 90 minutes. Immediately measure the absorbence relative to the control at 420 nm in a 10 mm cell.4.5. Plotting the calibration curve

Pipette 5,0, 10,0, 15,0 and 20 ml aliquots respectively into 4 × 50 ml volumetric flasks. Make up to the mark with sodium sulphate solution (10 %).

These solutions correspond to eluates obtained from the wine containing 0,5, 1,0, 1,5 and 2,0 g/l of malic acid.

Continue as in 4.4.

The graph of the absorbences of these solutions is a function of their malic acid concentration represented as a straight line passing through the origin.

The intensity of the colour produced depends to a large extent on the strength of the sulphuric acid used, it is necessary to check the calibration curve with at least one point per series of readings to check if the concentration of the sulphuric acid has changed.
5. EXPRESSION OF RESULTS

The concentration of the eluent is found using the calibration graph by extrapolation of the measured absorbence value to give the corresponding malic acid concentration in g/l. The result is expressed to one decimal place. Repeatability:

Contents < 2 g/l: r = 0,1 g/l.Contents > 2 g/l: r= 0,2 g/l. Reproducibility:

R = 0,3 g/l.

22. SORBIC ACID 1. PRINCIPLE OF METHODS

1.1. Determination by ultraviolet absorption spectrophotometry

Sorbic acid (trans, trans, 2,4-hexadienoic acid) extracted by steam distillation is determined in the wine distillate by ultraviolet absorption spectrophotometry. Substances that interfere in the ultraviolet are removed by evaporation to dryness using a lightly alkali, calcium hydroxide. Thin layer chromatography is used for confirmation of levels (1 mg/l) less than 20 mg/l.1.2. Determination by gas chromatography

Sorbic acid extracted in ethyl ether is determined by gas chromatography with an internal standard.1.3. Identification of traces by thin-layer chromatography

Sorbic acid extracted in ethyl ether is separated by thin layer chromatography and its concentration is evaluated semi-quantitatively.2. DETERMINATION BY ULTRAVIOLET ABSORPTION SPECTROPHOTOMETRY

2.1. Reagents

2.1.1. Crystalline tartaric acid, $C_4H_6O_6$.

2.1.2. Calcium hydroxide, $Ca(OH)_2$, solution, approximately 0,02 M.

2.1.3. Reference sorbic acid solution, 20 mg/l:

Dissolve 20 mg of sorbic acid, $C_6H_8O_2$, in approximately 2 ml of 0,1 M sodium hydroxide solution. Pour into a 1 000 ml volumetric flask, and make up to the mark with water. It is also possible to dissolve 26,8 mg of potassium sorbate, $C_6H_7KO_2$, in water and make up to 1 000 ml with water.2.2. Apparatus

2.2.1. Steam distillation apparatus (see chapter 'Volatile acidity').

2.2.2. Water bath at 100 °C.

2.2.3. Spectrophotometer enabling absorbence measurements to be made at a wavelength of 256 nm and having a quartz cell with a 1 cm optical path.2.3. Procedure

2.3.1. Distillation

Place in the flask of the steam distillation apparatus 10 ml of wine and add 1 to 2 g tartaric acid (2.1.1). Collect 250 ml of the distillate.2.3.2. Preparation of the calibration curve

Prepare, by dilution of the reference solution (2.1.3), four dilute reference solutions with 0,5, 1,0, 2,5 and 5 mg of sorbic acid per litre. Measure their absorbences with the spectro photometer at 256 nm using that of distilled water as a blank. Plot a curve showing the variation of absorbence as a function of concentration. The variation is linear.2.3.3. Determination

Place 5 ml of the distillate in an evaporating dish of 55 mm diameter, add 1 ml of calcium hydroxide solution (2.1.2). Evaporate to dryness on a water bath.

Dissolve the residue in several ml of distilled water, transfer completely to a 20 ml volumetric flask and make up to the mark with rinsing water. Measure the absorbence at 256 nm using the spectrophotometer against a blank consisting of a solution obtained by diluting 1 ml of calcium hydroxide solution (2.1.2) to 20 ml with water.

Plot the value of the measured absorbence on the calibration curve and from this find the concentration Cof sorbic acid in the solution.

Note: In this method the loss due to evaporation can be neglected and the absorbence measured on the treated distillate diluted ¼ with distilled water.2.4. Expression of results

2.4.1. Calculation

The sorbic acid concentration in the wine expressed in mg per litre is given by 100 × C

where C = concentration of sorbic acid in the solution analysed by spectrophotometry expressed in mg per litre.3. DETERMINATION BY GAS CHROMATOGRAPHY

3.1. Reagents

3.1.1. Ethyl ether, $(C_2H_5)_2O$, distilled just before use.

3.1.2. Internal reference solution: solution of undecanoic acid, $C_{11}H_{22}O_2$, in 95 % vol ethanol at a strength of 1 g/l.

3.1.3. Aqueous solution of sulphuric acid, H_2SO_4 (r_{20} = 1,84 g/ml) diluted 1:3 (v/v).3.2. Apparatus

3.2.1. Gas chromatograph fitted with a flame ionization detector and a stainless steel column (4 m × 1/8 inch) previously treated with dimethyldichlorosilane and packed with a stationary phase consisting of a mixture of diethyleneglycol succinate (5 %) and phosphoric acid (1 %) (DEGS - H_3PO_4) or of a mixture of diethyleneglycol adipate (7 %) and phosphoric acid (1 %) (DEGA - H_3PO_4) bonded on Gaschrom Q 80- 100 mesh.

Treatment of column with DMDCS - pass through the column a solution containing 2 to 3 g of DMDCS in toluene. Immediately wash with methanol, followed by nitrogen and then wash with hexane followed by more nitrogen. It is now ready to be packed.

Operating conditions:Oven temperature: 175 °C.Temperature of the injector and detector: 230 °C. Carrier gas: nitrogen (flow rate = 200 ml/min).

3.2.2. Microsyringe, 10 µl capacity graduated in 0,1 µl.

Note:Other types of columns that give a good separation can be used, particularly capillary columns (e.g. FFAP). The working method described is given as an example.3.3. Procedure

3.3.1. Preparation of sample to be analysed

Into a glass test tube of approximately 40 ml capacity and fitted with a ground glass stopper, introduce 20 ml of wine, add 2 ml of the internal reference solution (3.1.2) and 1 ml of dilute sulphuric acid (3.1.3).

After mixing the solution by repeatedly turning the tube over, add to its contents 10 ml of ethyl ether (3.1.1). Extract the sorbic acid in the organic phase by shaking the tube for five minutes. Leave to settle.3.3.2. Preparation of the reference solution

Select a wine for which the chromatogram of the ether extract shows no peak corresponding to the elution of sorbic acid. Overload this wine with sorbic acid at a concentration of 100 mg per litre. Treat 20 ml of the sample prepared in this way according to the procedure described in 3.3.1.3.3.3. Chromatography

Using a microsyringe, inject into the chromatograph in turn 2 µl of the ether-extract phase obtained in 3.3.2 and 2 µl of the ether-extracted phase obtained in 3.3.1.

Record the respective chromatograms: check the identity of the respective retention times of the sorbic acid and the internal standard. Measure the height (or area) of each of the recorded peaks.3.4. Expression of results

3.4.1. Calculation

The concentration of sorbic acid in the analysed wine, expressed in mg per litre, is given by:whereH = height of the sorbic acid peak in the reference solutionh = height of the sorbic acid peak in the sample for analysisI = height of the internal standard peak in the reference solutioni = height of the internal standard peak in the sample for analysis

Note:The sorbic acid concentration may be determined in the same way from measurements of the areas under the respective peaks.

4. IDENTIFICATION OF TRACES OF SORBIC ACID BY THIN LAYER CHROMATOGRAPHY

4.1. Reagents

4.1.1. Ethyl ether, (C2H5)2O.

4.1.2. Aqueous sulphuric acid solution, H2SO4 (r20 = 1,84 g/ml), diluted 1:3 (v/v).

4.1.3. Reference solution of sorbic acid in an approximately 10 % vol ethanol/water mixture containing 20 mg per litre.

4.1.4. Mobile phase: hexane-pentane-acetic acid (20:20:3) (C6H14/C5H12/CH3COOH, r20 = 1,05 g/ml).4.2. Apparatus

4.2.1. Precoated 20 × 20 cm plates for thin layer chromatography coated with polyamide gel (0,15 mm thick) with the addition of a fluorescent indicator.

4.2.2. Cell for thin layer chromatography.

4.2.3. Micropipette or microsyringe for delivering volumes of 5 µl to within ± 0,1 µl.

4.2.4. Ultraviolet lamp (254 nm).4.3. Procedure

4.3.1. Preparation of sample to be analysed

Into a glass test tube of approximately 25 ml capacity and fitted with a ground glass stopper, place 10 ml of wine, add 1 ml of dilute sulphuric acid (4.1.2) and 5 ml of ethyl ether (4.1.2). Mix by repeatedly turning the tube over. Leave to settle.4.3.2. Preparation of dilute reference solutions

Prepare five dilute reference solutions from the solution in 4.1.3 containing 2, 4, 6, 8 and 10 mg sorbic acid per litre.4.3.3. Chromatography

Using a microsyringe or micropipette, deposit 5 µl of the ether-extracted phase obtained in 4.3.1 and 5 µl of each of the dilute reference solutions (4.3.2) at points 2 cm from the lower edge of the plate and 2 cm apart from each other.

Place the mobile phase (4.1.4) in the chromatograph tank to a height of about 0,5 cm and allow the atmosphere in the tank to become saturated with solvent vapours. Place the plate in the tank. Allow the chromatogram to develop over 12 to 15 cm (development time approximately 30 minutes). Dry the plate in a current of cool air. Examine the chromatogram under a 254 nm ultraviolet lamp.

The spots indicating the presence of sorbic acid will appear to be dark violet against the yellow fluorescent background of the plate.4.4. Expression of results

A comparison of the intensities of the spots produced by the sample to be analysed and by the reference solutions will enable a semi-quantitative assessment to be made of the sorbic acid concentration between 2 and 10 mg per litre. A concentration of 1 mg per litre could be determined with the deposition of 10 µl of the sample solution to be analysed.Concentrations above 10 mg per litre could be determined with the deposition of less than 5 µl of the solution to be analysed (measured out using a microsyringe).

23. L-ASCORBIC ACID 1. PRINCIPLE OF METHODS

The methods proposed enable the L-ascorbic acid and dehydroascorbic acid present in wines or musts to be determined.1.1. Reference method (fluorimetry)

The L-ascorbic acid is oxidized on activated carbon into dehydroascorbic acid. The latter forms a fluorescent compound by reacting with orthophenylenediamine (OPDA). A control test in the presence of boric acid enables spurious fluorescence to be determined (by the formation of a boric acid/dehydroascorbic acid complex) and the fluorimetric determination to be deduced.1.2. Usual method (colorimetry)

The L-ascorbic acid is oxidized by iodine to dehydroascorbic acid which is then precipitated using 2,4-dinitrophenylhydrazine to produce bis (2,4-dinitrophenylhydrazone). After separation by thin layer chromatography and dissolution in acetic acid medium the red-coloured derivative is determined by spectrophotometry at 500 nm.2. REFERENCE METHOD (fluorimetric method)

2.1. Reagents

2.1.1. Orthophenylenediamine dihydrochloride solution, $C_6H_{10}Cl_2N_2$, 0,02 g per 100 ml, prepared just before use.

2.1.2. Sodium acetate trihydrate solution, $CH_3COONa \cdot 3H_2O$, 500 g/litre.

2.1.3. Mixed solution of boric acid and sodium acetate:dissolve 3 g of boric acid, H_3BO_3, in 100 ml of sodium acetate solution (2.1.2). This solution must be prepared just before use.

2.1.4. Glacial acetic acid solution, CH_3COOH (r_{20} = 1,05 g/ml), diluted to 56 % (v/v) with pH near to 1,2.

2.1.5. Reference solution of L-ascorbic acid, 1 g/litre:Just before use, dissolve 50 mg of L-ascorbic acid, $C_6H_8O_6$, previously dehydrated in a desiccator protected against light, in 50 ml of acetic acid solution (2.1.4).

2.1.6. Very pure analytical grade activated carbon (10) Into a 2-litre conical flask, place 100 g of activated carbon and add 500 ml of 10 % (v/v) hydrochloric acid (HCl) solution (r20 = 1,19 g/ml). Bring to the boil, filter using a sintered glass filter of porosity 3. Collect the carbon treated in this way in a 2-litre conical flask, add 1 litre of water, shake and filter using a sintered glass filter of porosity 3. Repeat this operation two more times. Place the residue in an oven controlled to 115 ± 5 °C for 12 hours (overnight).2.2. Apparatus

2.2.1. Fluorimeter. Use a spectrofluorimeter equipped with a lamp giving a continuous spectrum by using it at minimum power. The optimum excitation and emission wavelengths for the test will be determined beforehand and depend on the equipment used. As a guide, the excitation wavelength will be approximately 350 nm and the emission wavelength approximately 430 nm. Cells of 1 cm path length.

2.2.2. Sintered glass filter of porosity 3.

2.2.3. Test tubes (diameter approximately 10 mm).

2.2.4. Stirring rods for test tubes.2.3. Procedure

2.3.1. Preparation of the sample of wine or must

Take a volume of the wine or must and dilute to 100 ml in a graduated flask with the 56 % acetic acid solution (2.1.4) in order to obtain a solution with an L-ascorbic acid concentration between 0 and 60 mg/litre. Homogenize the contents of the flask by stirring. Add 2 g of activated carbon (2.1.6) and allow to stand for 15 minutes, stirring occasionally. Filter using ordinary filter paper, discarding the first few millilitres of filtrate.

Into two 100 ml graduated flasks, introduce 5 ml of the filtrate and, in the first, 5 ml of the mixed solution of boric acid and sodium acetate solution (2.1.3) (sample blank) and, in the second, 5 ml of the sodium acetate solution (2.1.2) (sample). Allow to stand for 15 minutes, stirring occasionally. Make up to 100 ml with distilled water.

Take 2 ml from the contents of each flask and add 5 ml of orthophenylenediamine solution (2.1.1), stir; leave the reaction to proceed for 30 minutes until the solution darkens and then make the spectrofluorimetric measurements.2.3.2. Preparation of the calibration curve

Into three 100 ml graduated flask place 2, 4 and 6 ml respectively of the reference L-ascorbic acid solution (2.1.5), make up to 100 ml with acetic acid solution (2.1.4) and homogenize by stirring. The reference solutions prepared in this way contain 2, 4 and 6 mg per 100 ml.

Add 2 g of activated carbon (2.1.6) to each of the flasks and allow to stand for 15 minutes, stirring occasionally. Filter through ordinary filter paper, discarding the first few millilitres. Introduce 5 ml of each filtrate collected into three 100-ml graduated flasks (first series). Repeat the operation and obtain a second series of three graduated flasks. To each of the flasks in the first series (corresponding to the blank test) add 5 ml of the mixed solution of boric acid and sodium acetate (2.1.5), and to each of the flasks in the second series add 5 ml of the sodium acetate solution (2.1.2).

Allow to stand for 15 minutes, stirring occasionally. Make up to 100 ml with distilled water. Take 2 ml of the contents of each flask, add 5 ml of orthophenylenediamine solution (2.1.1), stir, leave

the reaction to proceed for 30 minutes until the solution darkens and then make the spectrofluorimetric measurements.2.3.3. Fluorimetric determination

For each solution contributing to the calibration curve and for the solution to be determined set the zero on the scale of measurements using the corresponding control test sample. Then measure the intensity of the fluorescence for each solution over the calibration range and for the solution to be determined.

Plot the calibration curve, which should be a straight line passing through the origin. On this line, find the value relative to the determination and thus deduce the concentration C L-ascorbic acid + dehydroascorbic acid in the solution to be analysed.2.3.4. Expression of results

The concentration of L-ascorbic acid and dehydroascorbic acid in the wine in milligrams per litre is given by C × F, where Fis the dilution factor.

3. USUAL METHOD (colorimetric method)

3.1. Reagents

3.1.1. Metaphosphoric acid, 30 % (m/v) solution:take 30 g of metaphosphoric acid, (HPO3)n, previously crushed in a mortar. Wash rapidly by immersion in distilled water and stirring. Dissolve the washed acid in distilled water by shaking in a 100 ml graduated flask and make up to the reference mark. The resultant solution will have a concentration of approximately 30 % (m/v) of metaphosphoric acid.

3.1.2. 3 % (m/v) metaphosphoric acid solution prepared just before use by 1:10 dilution with distilled water of solution 3.1.1.

3.1.3. 1 % (m/v) metaphosphoric acid solution prepared just before use by 1:30 dilution with distilled water of solution 3.1.1.

3.1.4. Polyamide suspension:Make a suspension of 10 g of polyamide for chromatography with 60 ml of distilled water. Allow to stand for two hours. The quantity prepared is enough for four determinations.

3.1.5. Thiourea, (NH2)2CS.

3.1.6. Iodine solution, I2, 0,05 M.

3.1.7. 2,4-Dinitrophenylhydrazine solution, 6 % (m/v):Make a suspension of 6 g of 2,4-dinitrophenylhydrazine (C6H6N4O4) in 50 ml of glacial acetic acid (r20 = 1,05 g/ml) and add 50 ml of sulphuric acid (r20 = 1,84 g/ml). The 2,4-dinitrophenylhydrazine is dissolved by stirring.

3.1.8. Ethyl acetate (C4H8O2) with the addition of 2 % (v/v) glacial acetic acid (3.1.12).

3.1.9. Chloroform, CHCl3.

3.1.10. Aqueous starch solution, 0,5 % (m/v).

3.1.11. Mobile phase:ethyl acetate50 volchloroform60 volglacial acetic acid 5 vol

Leave the solvent mixture undisturbed for 12 hours before use.

3.1.12. Glacial acetic acid, CH3COOH, (r20 = 1,05 g/ml).

3.1.13. L-ascorbic acid solution, 0,1 g per 100 ml of 1 % metaphosphoric acid solution (3.1.3).3.2. Apparatus

3.2.1. Laboratory centrifuge having 50 ml centrifuge tubes with ground glass stoppers.

3.2.2. Cool waterbath, temperature thermostatically controlled between 5 and 10 °C.

3.2.3. Waterbath temperature thermostatically controlled to 20 °C.

3.2.4. Precoated plates for thin layer chromatography, 20 × 20 cm, coated with silica gel G (thickness 0,25 or 0,3 mm).

3.2.5. Chromatography tank.

3.2.6. Micropipette capable of delivering 0,2 ml volumes.

3.2.7. Spectrophotometer capable of making absorbence measurements at 500 nm equipped with cells having a 1 cm optical path.3.3. Procedure

3.3.1. Oxidation of the L-ascorbic acid to dehydroascorbic acid

Place 50 ml of the wine in a 100 ml volumetric flask, add 15 ml of the polyamide suspension (3.1.4) and make up to the mark with 3 % metaphosphoric acid solution (3.1.2). Allow to stand for one hour, shaking at frequent intervals. Filter through a fluted filter. Collect 20 ml of the filtrate in a centrifuge tube, add 1 ml of 0,05 M iodine solution (3.1.6). Homogenize by shaking the stoppered centrifuge tube and, after 1 minute, reduce the excess iodine by the addition of approximately 25 mg of thiourea.3.3.2. Formation and extraction of the bis(2,4-dinitrophenylhydrazone) derivative of diketogulonic acid

Place the tube into a waterbath with the temperature maintained to between 5 and 10 °C; add 4 ml of the 2,4-dinitrophenylhydrazine solution (3.1.7). Mix the contents carefully, avoiding any wetting of the glass stopper. Seal the tube completely and place in a waterbath at 20 °C for approximately 16 hours (overnight).

Introduce 15 ml of ethyl acetate (3.1.8) into the centrifuge tube. Close the tube with its ground glass stopper and shake vigorously for 30 seconds. Centrifuge for five minutes using a centrifugal force of 350 to 400 g. Pipette 10 ml of the ethyl acetate extract into a conical flask with a ground glass stopper. Remove stopper and add a further 5 ml of ethyl acetate (3.1.8) to the centrifuge tube, again shake for 30 seconds and centrifuge for five minutes at the same speed. Pipette 5 ml of the ethyl acetate extract into the conical flask containing the 10 ml from the first extraction. Mix.3.3.3. Separation of the bis(2,4-dinitrophenylhydrazone) by chromatography; this is to be carried out in the 2 hours following the extraction (3.3.2).Apply 0,2 ml of the ethyl acetate extract to the whole of a starting line situated 2 cm from the edge of the plate, leaving a margin of 2 cm at the sides of the plate. Place the mobile phase (3.1.11) in the chromatography tank to a depth of

1 cm and allow the atmosphere to become saturated with solvent vapours. Introduce the plate, allowing the solvent to run to the top edge of the plate.

Dry the plate for one hour under a ventilated hood. Hold the plate in a vertical position above a sheet of glazed paper and with a spatula scrape off (at right angles to the direction of the run) the red-coloured zone (characteristic of the 2,4-dinitrophenylhydrazone). Transfer the powdered product obtained, without loss, to a conical flask fitted with a ground glass stopper, and add 4 ml of acetic acid (3.1.12). Allow to stand for 30 minutes, stirring frequently. Filter through a fluted filter paper directly into a spectrophotometric cell (3.2.7). The filtrate obtained must be perfectly clear. Measure the absorbence of this solution at 500 nm using acetic acid (3.1.12) in the reference cell to provide a zero for the measurements.3.3.4. Preparation of the calibration curve

Place 5, 10 and 15 ml of the L-ascorbic acid solution (3.1.13) in three 100 ml volumetric flasks and make up to the mark with the 1 % metaphosphoric acid solution (3.1.3). The solutions thus obtained have concentrations of 50, 100 and 150 mg of ascorbic acid per litre respectively.

Treat each of these solutions according to the procedure described in 3.3.1, 3.3.2 and 3.3.3. Plot the calibration curve, which should be a straight line passing through the origin.3.3.5. Expression of results

The concentration of L-ascorbic acid + dehydroascorbic acid in the wine is expressed in milligrams per litre.
3.3.5.1. Calculation

Find the value of the absorbence measured in 3.3.3 on the straight calibration curve and thus determine the concentration of L-ascorbic acid + dehydroascorbic acid in the solution to be analysed.

Note: If the concentration of L-ascorbic acid + dehydroascorbic acid is higher than 150 mg/litre, reduce the volume of the test sample to 25, 20 or 10 ml of wine and multiply the result obtained by the dilution factor F.

24. pH 1. PRINCIPLE

The difference in potential between two electrodes immersed in the liquid under test is measured. One of these two electrodes has a potential which is a function of the pH of the liquid, while the other has a fixed and known potential and constitutes the reference electrode.2. APPARATUS

2.1. pH meter with a scale calibrated in pH units and enabling measurements to be made to at least ±0,05 pH unit.

2.2. Electrodes:

2.2.1. Glass electrode, kept in distilled water.

2.2.2. Calomel-saturated potassium chloride reference electrode, kept in a saturated solution of potassium chloride.

2.2.3. Or a combined electrode, kept in distilled water.3. REAGENTS

3.1. Buffer solutions

3.1.1. Saturated solution of potassium hydrogen tartrate, containing at least 5,7 g of potassium hydrogen tartrate per litre (C4H5KO6) at 20 °C. (This solution may be kept for up to two months by adding 0,1 g of thymol per 200 ml.)

pH 3,57 at 20 °CpH 3,56 at 25 °CpH 3,55 at 30 °C

3.1.2. Solution of potassium hydrogen phthalate, 0,05 M, containing 10,211 g of potassium hydrogen phthalate (C8H5KO4) per litre at 20 °C. (Maximum keeping period, two months.)pH 3,999 at 15 °CpH 4,003 at 20 °CpH 4,008 at 25 °CpH 4,015 at 30 °C

3.1.3. Solution containing: monopotassium phosphate, KH2PO43,402 gdipotassium phosphate, K2HPO44,354 gwater to1 000 ml (maximum keeping period, two months)pH 6,90 at 15 °CpH 6,88 at 20°CpH 6,86 at 25 °CpH 6,85 at 30 °C

Note: Commerical reference buffer solutions may also be used.
4. PROCEDURE

4.1. Preparation of the sample for analysis

4.1.1. For must and wine: use the must or wine directly.

4.1.2. For rectified concentrated must: dilute the rectified concentrated must with water to produce a concentration of 25 ± 0,5 % (m/m) of total sugars (25 ° Brix).

If P is the percentage concentration (m/m) of total sugars in the rectified concentrated must, weigh a mass ofand make up to 100 g with water. The water used must have a conductivity below 2 microsiemens per cm.4.2. Zeroing of the apparatus

Zeroing is carried out before any measurement is made, according to the instructions provided with the apparatus used.4.3. Calibration of the pH meter

Calibrate the pH meter at 20 °C using buffer solutions of pH 6,88 and 3,57 at 20 °C.

Use the buffer solution of pH 4,00 at 20 °C to check the calibration of the scale.4.4. Determination

Dip the electrode into the sample to be analysed, the temperature of which should be between 20 and 25 °C and as close as possible to 20 °C. Read the pH value directly off the scale.

Carry out at least two determinations on the same sample.

The final result is taken to be the arithmetic mean of two determinations.5. EXPRESSION OF RESULTS

The pH of the must, the wine or the 25 % (m/m) (25 ° Brix) solution of rectified concentrated must is quoted to two decimal places.

25. SULPHUR DIOXIDE 1. DEFINITIONS

Free sulphur dioxide is defined as the sulphur dioxide present in the must or wine in the following forms: H2SO3, HSO3

The equilibrium between these forms is a function of pH and temperature:H2SO3 H+ + HSO3 H2SO3 represents molecular sulphur dioxide.

Total sulphur dioxide is defined as the total of all the various forms of sulphur dioxide present in the wine, either in the free state or combined with its constituents.2. FREE AND TOTAL SULPHUR DIOXIDE

2.1. Principle of the methods

2.1.1. Reference method

2.1.1.1. For wines and musts

The sulphur dioxide is carried over by a current of air or nitrogen; it is fixed and oxidized by being bubbled through a dilute and neutral hydrogen peroxide solution. The sulphuric acid formed is determined by titration with a standard solution of sodium hydroxide. Free sulphur dioxide is purged from the wine by entrainment at low temperature (10 °C).

Total sulphur dioxide is purged from the wine by entrainment at high temperature (approximately 100 °C).2.1.1.2. For rectified concentrated musts

Total sulphur dioxide is extracted from the previously diluted rectified concentrated must by entrainment at high temperature (approximately 100 °C).2.1.2. Rapid method of determination (for wines and musts)

Free sulphur dioxide is determined by direct iodometric titration.

Combined sulphur dioxide is subsequently determined by iodometric titration after alkaline hydrolysis. When added to the free sulphur dioxide, it gives the total sulphur dioxide.2.2. Reference method

2.2.1. Apparatus

2.2.1.1. The apparatus used should conform to the diagram shown below, particularly with regard to the condenser.

The dimensions given are in millimetres. The internal diameters of the four concentric tubes forming the condenser are 45, 34, 27 and 10 mm.

The gas feed tube to the bubbler B ends in a small sphere of 1 cm diameter with 20 0,2-mm diameter holes around its largest horizontal circumference. Alternatively, this tube may end in a frit glass plate which produces a large number of very small bubbles and thus ensures good contact between the liquid and gaseous phases.

The gas flow through the apparatus should be approximately 40 litres per hour. The bottle on the right of the diagram is intended to restrict the pressure reduction produced by the water pump to 20 to 30 cm of water. To regulate the vacuum to its correct value, a flowmeter with a semi-capillary tube should be installed between the bubbler and the bottle.

2.2.1.2. A microburette.2.2.2. Reagents

2.2.2.1. Phosphoric acid, 85 % (H_3PO_4, r20 = 1,71 g/ml).

2.2.2.2. Hydrogen peroxide solution, 9,1 g H_2O_2/litre (three volumes).

2.2.2.3. Indicator reagent: methyl red100 mgmethylene blue 50 mgalcohol, 50 % vol100 ml

2.2.2.4. Sodium hydroxide solution, NaOH, 0,01 M

2.2.3. Procedure

2.2.3.1. Determination of free sulphur dioxide

The wine must be kept in a full and stoppered bottle at 20 °C for two days before the determination.Place 2 to 3 ml of hydrogen peroxide solution (2.2.2.2) and two drops of the indicator reagent in the bubbler B and neutralize the hydrogen peroxide solution with the 0,01 M sodium hydroxide solution (2.2.2.4). Connect the bubbler to the apparatus.Introduce 50 ml of the sample and 15 ml of phosphoric acid (2.2.2.1) into the flask A of the entrainment apparatus. Connect the flask into the apparatus.Bubble air (or nitrogen) through it for 15 minutes. The free sulphur dioxide carried over is oxidized to sulphuric acid. Remove the bubbler from the apparatus and titrate the acid which has formed against the 0,01 M sodium hydroxide solution (2.2.2.4). Let n ml be the volume used.2.2.3.2. Expression of results

The liberated sulphur dioxide is expressed in mg/l to the nearest whole number.

2.2.3.2.1. Calculation

The free sulphur dioxide in milligrams per litre is 6,4 n.2.2.3.3. Determination of total sulphur dioxide

2.2.3.3.1. For rectified concentrated musts, use the solution obtained by diluting the sample to be analysed to 40 % (m/v) as indicated in the chapter 'Total acidity', section 5.1.2. Introduce 50 ml of this solution and 5 ml of phosphoric acid (2.2.2.1) into the 250 ml flask A of the entrainment apparatus. Connect the flask into the apparatus.

2.2.3.3.2. Wines and musts

If the estimated concentration in the sample is no greater than 50 mg of total SO_2 per litre, place 50 ml of the sample and 15 ml of phosphoric acid (2.2.2.1) in the 250-ml flask A of the entrainment apparatus. Connect the flask to the apparatus.

However, until 31 December 1992 at the latest, to analyse the sulphur dioxide content of grape juice, 5 ml of a 25 % solution (m/v) of phosphoric acid (2.2.2.1) shall be used.

If the estimated concentration in the sample is greater than 50 mg of total SO2 per litre, place 20 ml of the sample and 5 ml of phosphoric acid (2.2.2.1) in the 100 ml flask A of the entrainment apparatus. Connect the flask to the apparatus.

Place 2 to 3 ml of hydrogen peroxide solution (2.2.2.2) in the bubbler B, neutralized as before, and bring the wine in the flask A to the boil using a small flame of 4 to 5 cm height which should directly touch the bottom of the flask. Do not put the flask on a metal plate but on a disc with a hole of approximately 30 mm diameter in it. This is to avoid overheating substances extracted from the wine that are deposited on the walls of the flask.

Maintain boiling while passing a current of air (or nitrogen). Within 15 minutes the total sulphur dioxide has been carried over and oxidized. Determine the sulphuric acid which has formed by titration with the 0,01 M sodium hydroxide solution (2.2.2.4).

Let n ml be the volume used.2.2.3.4. Expression of results

Musts and wines: Total sulphur dioxide is expressed in mg/l.

Rectified concentrated must: Total sulphur dioxide is expressed in mg/kg of total sugar.

2.2.3.4.1. Calculation

For wines:Total sulphur dioxide in milligrams per litre:- samples low in sulphur dioxide (50 ml test sample): 6,4 n- other samples (20 ml test sample): 16 nFor rectified concentrated musts:Total sulphur dioxide in milligrams per kilogram of total sugars (50 ml prepared test sample (2.2.3.3.1)):where P = percentage concentration (m/m) of total sugars

2.2.3.4.2. Repeatability (r)50 ml test sample < 50 mg/l; r = 1 mg/l.20 ml test sample > 50 mg/l; r = 6 mg/l.

2.2.3.4.3. Reproducibility (R)50 ml test sample < 50 mg/l; R = 9 mg/l.20 ml test sample > 50 mg/l; R = 15 mg/l.2.3. Rapid method of determination

2.3.1. Reagents

2.3.1.1. EDTA Complexone III: disodium salt of ethene diamine tetra-acetic acid ($C_{10}H_{14}N_2O_8Na_2 \cdot 2H_2O$)

2.3.1.2. Sodium hydroxide solution, NaOH, 4 M (160 g/l).

2.3.1.3. Sulphuric acid, H_2SO_4 (r20 = 1,84 g/ml) (1:10 v/v).

2.3.1.4. Starch solution 5 g/l:

Mix 5 g starch with about 500 ml of water. Bring to the boil stirring continuously and keep boiling for 10 minutes. Add 200 g of sodium chloride (NaCl). Make up to one litre after cooling.

2.3.1.5. 0,025 M iodine solution, I2.2.3.2. Apparatus

2.3.2.1. 500 ml conical flasks.

2.3.2.2. Burette.

2.3.2.5. 1, 2, 5 and 50 ml pipettes.2.3.3. Procedure

2.3.3.1. Free sulphur dioxide

Place in a 500 ml conical flask:- 50 ml wine,- 5 ml starch solution (2.3.1.4),- 30 mg EDTA Complexone III (2.3.1.1),- 3 ml of 1/10 (v/v) H_2SO_4 (2.3.1.3).

Titrate immediately with 0,025 M iodine (2.3.1.5) until the blue coloration persists clearly for 10 to 15 seconds. Let n ml be the volume of iodine used.2.3.3.2. Sulphur dioxide

Add 8 ml of 4 M sodium hydroxide solution (2.3.1.2), shake the mixture once and allow to stand for five minutes. Add, with vigorous stirring and in one operation, the contents of a small beaker in which 10 ml of 1:10 v/v sulphuric acid (2.3.1.3) have been placed. Titrate immediately with 0,025 M iodine (2.3.1.5); let the volume used be n2 ml.

Add 20 ml of 4 M sodium hydroxide solution (2.3.1.2), shake once and allow to stand for five minutes. Dilute with 200 ml of ice-cold water.

Add, with vigorous stirring and in one operation, the contents of a test tube in which 30 ml of 1:10 v/v sulphuric acid (2.3.1.3) have previously been placed. Titrate the free sulphur dioxide immediately with 0,025 M iodine (2.3.1.5), and let the volume used be n" ml.2.3.4. Expression of results

2.3.4.1. Calculation:

Free sulphur dioxide in milligrams per litre: 32 n.Total sulphur dioxide in milligrams per litre: 32 (n + n2+ n").

Notes:(1) For red wines with low SO_2 concentrations, it is worth using iodine more dilute than 0,025 M (e.g. 0,01 M. Then replace the coefficient 32 by 12,8 in the above formulae).
(2) For red wines, it is useful to illuminate from below with a beam of yellow light from an ordinary electric light bulb shining through a solution of potassium chromate or from a sodium vapour lamp. The determination should be carried out in a dark room and the transparency of the wine observed: it becomes opaque when the end-point is reached.(3) If the quantity of sulphur dioxide (H_2SO_3) is close to, or exceeds, the legal limit, the total sulphur dioxide should be determined by the reference method.(4) If the determination of free sulphur dioxide is particularly required, carry out the analysis on a sample kept under anaerobic conditions for two days at 20 °C before analysis. Carry out the determination at 20 °C.(5) Because certain substances are oxidized by iodine in an acid medium, the quantity of iodine used in this way must be evaluated for more accurate determinations. To achieve this, the free sulphur dioxide should be combined with excess ethanol or propanal before carrying out the iodine titration. Add 5 ml of 7 g/l ethanol solution (C_2H_4O) or 5 ml of a 10 g/l propanal solution (C_3H_6O) to 50 ml of wine in a 300 ml conical flask.Put a stopper on the flask and leave standing for at least 30 minutes. Add 3 ml of 1:10 v/v sulphuric acid (2.3.1.3) and sufficient 0,025 M iodine (2.3.1.5) to cause the starch to change colour. Let n4 ml be the volume of iodine used. This must be subtracted from n (free sulphur dioxide) and from n + n2 + n" (total sulphur dioxide).n4 is generally small, from 0,2 to 0,3 ml 0,025 M iodine. If ascorbic acid has been added to the wine, n4 will be much higher and it

is possible, at least approximately, to measure the amount of this substance from the value of n4, given that 1 ml of 0,025 M iodine will oxidize 4,4 mg of ascorbic acid. By determining n4 it is possible to detect quite easily the presence of residual ascorbic acid in amounts greater than 20 mg/l in wines to which it has been added.3. MOLECULAR SULPHUR DIOXIDE

3.1. Principle of the method

The percentage of molecular sulphur dioxide, H2SO3, in free sulphur dioxide is calculated as a function of pH, alcoholic strength and temperature.

For a given temperature and alcoholic strength:H2SO3 H+ + HSO3 ,whereI = ionic strength,A and B = coefficients varying with temperature and alcoholic strength,KT = thermodynamic dissociation constant: values of pKTare given in Table 1 for various alcoholic strengths and temperatures,KM = mixed dissociation constant.

Taking a mean value of 0,038 for the ionic strength I,Table 2 gives values of pKM for various temperatures and alcoholic strengths.

The molecular sulphur dioxide content calculated using expression (1) is given in Table 3 for various values of pH, temperature and alcoholic strength.3.2.Calculation

From a knowledge of the pH of the wine and its alcoholic strength, the percentage of molecular sulphur dioxide is given in Table 3 for a temperature T °C. Let this be X %.

The molecular sulphur dioxide content in mg/l is:X × Cwhere C = the free sulphur dioxide content in mg/l.
TABLE 1Values of the thermodynamic dissociation constant pKT

TABLE 2Values of the mixed dissociation constant pKM (I = 0,038)

TABLE 3Molecular sulphur dioxide as a percentage of free sulphur dioxide

TABLE 3(continued)

25. SODIUM 1. PRINCIPLE OF THE METHODS

1.1. Reference method: atomic absorption spectrophotometry

Sodium is determined directly in the wine by atomic absorption spectrophotometry after the addition of an ionization suppression agent (caesium chloride) to prevent ionization of sodium.1.2. Usual method: flame photometry

Sodium is determined directly in diluted wine (at least 1:10) by flame photometry.2. REFERENCE METHOD

2.1. Reagents

2.1.1. Solution containing 1 g of sodium per litre:

Use a standard commercial solution containing 1 g of sodium per litre. This solution may be prepared by dissolving 2,542 g of anhydrous sodium chloride, NaCl, in distilled water and making up to a volume of 1 litre.

Keep this solution in a polyethylene bottle.2.1.2. Matrix (model) solution:

citric acid, C6H8O7 · H2O 3,5 gsucrose, C12H22O11 1,5 gglycerol, C3H8O3 5,0 ganhydrous calcium chloride, CaCl2 50 mganhydrous magnesium chloride, MgCl2 50 mgabsolute alcohol, C2H5OH 50 mlde-ionized water to500 ml2.1.3. Caesium chloride solution containing 5 % caesium:

dissolve 6,330 g of caesium chloride, CsCl, in 100 ml of distilled water.2.2. Apparatus

2.2.1. Atomic absorption spectrophotometer equipped with an air-acetylene burner.

2.2.2. Sodium hollow cathode lamp.2.3. Procedure

2.3.1. Preparation of sample

Pipette 2,5 ml of wine into a 50 ml volumetric flask, add 1 ml of the caesium chloride solution (2.1.3) and make up to the mark with distilled water.
2.3.2. Calibration

Place 5,0 ml of the matrix solution in each one of a set of 100 ml volumetric flasks and add 0, 2,5, 5,0, 7,5 and 10 ml respectively of the 1 g/l sodium solution (2.1.1) previously diluted by 1:100. Add 2 ml of the caesium chloride solution (2.1.3) to each flask and make up to 100 ml with distilled water.

The standard solutions prepared in this way contain 0, 0,25, 0,50, 0,75 and 1,00 mg of sodium per litre respectively and each contains 1 g of caesium per litre. Keep these solutions in polyethylene bottles.2.3.3. Determination

Set the wavelength to 589,0 nm. Zero the absorbence scale using the matrix solution containing 1 g of caesium per litre (2.3.2). Aspirate the diluted wine directly into the burner of the spectrophotometer, followed in succession by the standard solutions (2.3.2). Read off the absorbences. Repeat each measurement.2.4. Expression of results

2.4.1.Method of calculation

Plot a graph giving the absorbence as a function of the sodium concentration in the standard solutions.

Record the absorbence obtained with the diluted wine on this graph and determine its sodium concentration C in milligrams per litre.

The sodium concentration in milligrams per litre of the wine will then be 20C, expressed to the nearest whole number.2.4.2. Repeatability (r)

r = 1 + 0,024 xi mg/l.xi = concentration of sodium in the sample in mg/l.2.4.3.Reproducibility (R)

R = 2,5 + 0,05 ximg/l.xi = concentration of sodium in the sample in mg/l.3. USUAL METHOD

3.1. Reagents

3.1.1. Reference solution containing 20 mg sodium per litre

Absolute alcohol (C2H5OH) 10 mlCitric acid (C6H8O7 · H2O) 700 mgSucrose (C12H22O11) 300 mgGlycerol (C3H8O3)1 000 mgPotassium hydrogen tartrate (C4H5KO6) 481,3 mgAnhydrous calcium chloride (CaCl2) 10 mgAnhydrous magnesium chloride (MgCl2) 10 mgDry sodium chloride (NaCl) 50,84 mgWater to 1 litre
3.1.2. Dilution solution

Absolute alcohol (C2H5OH) 10 mlCitric acid (C6H8O7 · H2O) 700 mgSucrose (C12H22O11) 300 mgGlycerol (C3H8O3)1 000 mgPotassium hydrogen tartrate (C4H5KO6) 481,3 mgAnhydrous calcium chloride (CaCl2) 10 mgAnhydrous magnesium chloride (MgCl2) 10 mgWater to 1 litre

To prepare solution 3.1.1 and 3.1.2 dissolve the potassium hydrogen tartrate in approximately 500 ml of very hot distilled water, mix this solution with 400 ml of distilled water in which the other chemicals have already been dissolved, and make up to one litre.

Preserve the solutions in polyethylene bottles by adding two drops of allyl isothiocyanate.3.2. Apparatus

3.2.1. Flame photometer supplied by an air-butane mixture.3.3. Procedure

3.3.1. Calibration

Place 5, 10, 15, 20 and 25 ml of the reference solution (3.1.1) in 100-ml volumetric flasks and make up to 100 ml with the dilution solution (3.1.2). Solutions containing 1, 2, 3, 4 and 5 mg of sodium per litre respectively are obtained in this way.3.3.2. Determination

Make the measurements at 589,0 nm. Carry out the adjustment to 100 % transmission using distilled water. Successively aspirate the standard solutions (3.3.1) directly into the burner of the photometer, followed by the wine diluted by 1:10 with distilled water and note the readings of percentage transmission. If necessary, the wine already diluted 1:10 may be further diluted with the dilution solution (3.1.2).3.4. Expression of results

3.4.1. Method of calculation

Plot a graph showing the variations in percentage transmission with sodium concentration in the standard solutions. Record the transmission obtained with the sample of diluted wine from this graph and determine the corresponding sodium concentration C.

The sodium concentration in mg of sodium per litre will be F×C where Fis the dilution factor.3.4.2. Repeatability (r)

r = 1,4 mg/l (except for liqueur wine)r = 2,0 mg/l for liqueur wine.3.4.3. Reproducibility (R)

R = 4,7 + 0,08 xi mg/l.xi = sodium concentration in the sample in mg/l.

27. POTASSIUM 1. PRINCIPLE OF THE METHODS

1.1. Reference method

Potassium is determined directly in the diluted wine by atomic absorption spectrophotometry after the addition of an ionization suppression agent (caesium chloride) to prevent ionization of potassium.1.2. Usual method

Potassium is determined directly in the diluted wine by flame photometry.2. REFERENCE METHOD

2.1. Reagents

2.1.1. Solution containing 1 g of potassium per litre:

Use a standard commercial solution containing 1 g of potassium per litre. This solution may be prepared by dissolving 4,813 g of potassium hydrogen tartrate ($C_4H_5KO_6$) in distilled water and making up the volume to 1 litre.

2.1.2. Matrix (model) solution:

citric acid ($C_6H_8O_7 \cdot H_2O$) 3,5 gsucrose ($C_{12}H_{22}O_{11}$) 1,5 gglycerol ($C_3H_8O_3$) 5,0 ganhydrous calcium chloride ($CaCl_2$) 50 mganhydrous magnesium chloride ($MgCl_2$) 50 mgabsolute alcohol (C_2H_5OH) 50 mlwater to500 ml2.1.3. Caesium chloride solution containing 5 % caesium:

dissolve 6,33 g of caesium chloride, CsCl, in 100 ml of distilled water.2.2. Apparatus

2.2.1. Atomic absorption spectrophotometer, equipped with an air-acetylene burner.

2.2.2. Potassium hollow cathode lamp.2.3. Procedure

2.3.1. Preparation of sample

Pipette 2,5 ml of wine (previously diluted by 1:10) into a 50-ml volumetric flask, add 1 ml of the caesium chloride solution (2.1.3) and make up to the mark with distilled water.2.3.2. Calibration

Introduce 5,0 ml of the matrix solution (2.1.2) into each one of a set of 100-ml volumetric flasks and add 0, 2,0, 4,0, 6,0 and 8,0 ml respectively of the 1 g/l potassium solution (2.1.1)

(previously diluted by 1:10). Add 2 ml of the caesium chloride solution (2.1.3) to each flask and make up to 100 ml with distilled water.

The standard solutions prepared in this way contain 0, 2, 4, 6 and 8 mg of potassium per litre respectively and each contains 1 g of caesium per litre. Keep these solutions in polyethylene bottles.2.3.3. Determination

Set the wavelength to 769,9 nm. Zero the absorbence scale using the matrix solution containing 1 g of caesium per litre (2.3.2). Aspirate the diluted wine (2.3.1) directly into the burner of the spectrophotometer, followed in succession by the standard solutions (2.3.2). Read off the absorbences. Repeat each measurement.2.4. Expression of results

2.4.1. Method of calculation

Plot a graph giving the variation in absorbence as a function of the potassium concentration in the standard solutions.

Record the mean value of the absorbence obtained with the sample of diluted wine on this graph and determine its potassium concentration Cin milligrams per litre.

The potassium concentration expressed in milligrams per litre of the wine to the nearest whole number will then be F×C, where F is the dilution factor (here 200).2.4.2. Repeatability (r)r = 35 mg/l.

2.4.3. Reproducibility (R)R = 66 mg/l.2.4.4. Other ways of expressing results

In milliequivalents per litre: 0,0256 × F × C. In mg potassium hydrogen tartrate per litre: 4,813 × F × C.3. USUAL METHOD: FLAME PHOTOMETRY

3.1. Reagents

3.1.1. Reference solution containing 100 mg potassium per litre

Absolute alcohol (C2H5OH) 10 mlCitric acid (C6H8O7×H2O) 700 mgSucrose (C12H22O11) 300 mgGlycerol (C3H8O3)1 000 mgSodium chloride (NaCl) 50,8 mgAnhydrous calcium chloride (CaCl2) 10 mgAnhydrous magnesium chloride (MgCl2) 10 mgDry potassium hydrogen tartrate (C4H5KO6) 481,3 mgWater to1 000 ml

Dissolve the potassium hydrogen tartrate in 500 ml of very hot distilled water, mix this solution with 400 ml of distilled water in which the other chemicals have already been dissolved, and make up to one litre.
3.1.2. Dilution solution

Absolute alcohol (C2H5OH) 10 mlCitric acid (C6H8O7 · H2O 700 mgSucrose (C12H22O11) 300 mgGlycerol (C3H8O3)1 000 mgSodium chloride (NaCl) 50,8 mgAnhydrous calcium chloride (CaCl2) 10 mgAnhydrous magnesium chloride (MgCl2) 10 mgTartaric acid (C4H6O6) 383 mgWater to1 000 ml

Preserve the solutions in polyethylene bottles by adding two drops of allyl isothiocyanate.3.2. Apparatus

3.2.1. Flame photometer supplied by an air-butane mixture.3.3. Procedure

3.3.1. Calibration

Place 25, 50, 75 and 100 ml of the reference solution (3.1.1) into a set of 100 ml volumetric flasks and make up to 100 ml with the dilution solution (3.1.2). Solutions containing 25, 50, 75 and 100 mg of potassium per litre respectively are obtained in this way.3.3.2. Determination

Make the measurements at 766 nm. Carry out the adjustment to 100 % transmission using distilled water. Successively aspirate the standard solutions (3.3.1) directly into the burner of the photometer, followed by the wine diluted 1:10 with distilled water and note the readings. If

necessary, the wine already diluted 1:10 may be further diluted with the dilution solution (3.1.2).3.4. Expression of results

3.4.1. Method of calculation

Plot the graph of the variation in percentage transmission as a function of the potassium concentrations in the standard solutions. Record the transmission obtained for the sample of diluted wine on this graph and determine the corresponding potassium concentration C.

The potassium concentration in mg potassium per litre to the nearest whole number will be F×C, where F is the dilution factor:3.4.2. Repeatability (r)

r = 17 mg/l.3.4.3. Reproducibility (R)

R = 66 mg/l.
3.4.4. Other ways of expressing results:

In milliequivalents per litre: 0,0256 × F × C. In mg potassium hydrogen tartrate per litre: 4,813 × F × C.

28. MAGNESIUM 1. PRINCIPLE OF THE METHOD

Magnesium is determined directly on wine, suitably diluted, by atomic absorption spectrophotometry.2. REAGENTS

2.1. Concentrated standard solution containing 1 g magnesium per litre

Use a standard commercial magnesium solution (1 g/l). This solution may be prepared by dissolving 8,3646 g of magnesium chloride (MgCl2.6H2O) in distilled water and making up to 1 litre.2.2. Dilute standard solution containing 5 mg magnesium per litre.

Note: Keep the standard magnesium solutions in polyethylene bottles.3. APPARATUS

3.1. Atomic absorption spectrophotometer fitted with an air-acetylene burner.

3.2. Magnesium hollow cathode lamp.4. PROCEDURE

4.1. Preparation of sample

Dilute the wine by 1:100 with distilled water.4.2. Calibration

Place 5, 10, 15 and 20 ml of the dilute standard magnesium solution (2.2) into each one of a set of 100 ml volumetric flasks and make up to 100 ml with distilled water. The standard solutions prepared in this way contain 0,25, 0,50, 0,75 and 1,0 mg of magnesium per litre respectively. These solutions should be kept in polyethylene bottles.4.3. Determination

Set the wavelength to 285 nm. Zero the absorbence scale using distilled water. Aspirate the diluted wine directly into the burner of the spectrophotometer, followed in succession by the standard solutions (4.2).

Read off the absorbences. Repeat each measurement.5. EXPRESSION OF RESULTS

5.1. Method of calculation

Plot a graph of the variation in absorbence as a function of the magnesium concentration in the standard solutions. Record the mean value of the absorbence obtained with the diluted sample of wine on this graph and determine its magnesium concentration C in milligrams per litre.

The magnesium concentration in milligrams per litre of the wine to the nearest whole number will be 100 C.
5.2.Repeatability (r)

r = 3 mg/l.5.3. Reproducibility (R)

R = 8 mg/l.

29. CALCIUM 1. PRINCIPLE OF THE METHOD

Calcium is determined directly on wine, suitably diluted, by atomic absorption spectrophotometry, after addition of an ionization suppression agent.2. REAGENTS

2.1.Standard solution containing 1 g calcium per litre

Use a standard commercial calcium solution 1 g/l. This solution may be prepared by dissolving 2,5 g of calcium carbonate, $CaCO_3$, in a quantity of 1:10 (v/v) HCl sufficient to dissolve it completely and making up to one litre with distilled water.2.2.Dilute standard solution containing 50 mg calcium per litre

Note: Keep the standard calcium solutions in polyethylene bottles.2.3. Lanthanum chloride solution containing 50 g lanthanum per litre

Dissolve 13,369 g of lanthanum chloride, $LaCl_3 \cdot 7H_2O$, in distilled water; add 1 ml of HCl diluted 1:10 (v/v) and make up to 100 ml.3. APPARATUS

3.1. Atomic absorption spectrophotometer fitted with an air-acetylene burner.

3.2. Calcium hollow cathode lamp.4. PROCEDURE

4.1. Preparation of sample

Place 1 ml of the wine, 2 ml of the lanthanum chloride solution (2.3) in a 20 ml volumetric flask and make up to the mark with distilled water. The wine, diluted by 1:20, contains 5 g lanthanum per litre.

Note: For sweet wines, the concentration of 5 g lanthanum per litre is sufficient provided the dilution does not bring the sugar content to below 2,5 g per litre. For wines with higher concentrations of sugar, the lanthanum concentration should be increased to 10 g per litre.4.2. Calibration

Place 0, 5, 10, 15 and 20 ml of the dilute standard calcium solution (2.2) respectively into a set of 100 ml volumetric flasks, add to each flask 10 ml of the lanthanum chloride solution (2.3) and make up to 100 ml with distilled water. The solutions prepared in this way contain 0, 2,5, 5,0, 7,5 and 10 mg of calcium per litre respectively and each contains 5 g of lanthanum per litre. These solutions should be kept in polyethylene bottles.4.3. Determination

Set the wavelength to 422,7 nm. Zero the absorbence scale using the solution containing 5 g of lanthanum per litre (4.2). Aspirate the diluted wine directly into the burner of the spectrophotometer, followed in succession by the five standard solutions (4.2). Read the absorbences. Repeat each measurement.
5. EXPRESSION OF RESULTS

5.1. Method of calculation

Plot a graph giving the variation in absorbence as a function of the calcium concentration in the standard solutions.

Record the mean value of the absorbence obtained with the sample of diluted wine on this graph and determine its calcium concentration C. The calcium concentration in milligrams per litre of the wine to the nearest whole number will be 20 C.5.2. Repeatability (r)

Concentration < 60 mg/l: r = 2,7 mg/l.Concentration > 60 mg/l: r = 4 mg/l.5.3. Reproducibility (R)

R = 0,114xi 0,5.xi = concentration in the sample in mg/l.

30. IRON 1. PRINCIPLE OF THE METHODS

Reference method

After suitable dilution of the wine and removal of alcohol, iron is determined directly by atomic absorption spectrophotometry. Usual method

After digestion in 30 % hydrogen peroxide solution, the total iron, now in the Fe(III) state, is reduced to the Fe(II) state and is determined using the coloration produced by orthophenanthroline.2. REFERENCE METHOD

2.1. Reagents

2.1.1. Concentrated standard iron solution containing 1 g Fe(III) per litre.

Use a standard commercial solution (1 g/l). This solution may be prepared by dissolving 8,6341 g of ferric ammonium sulphate ($FeNH_4(SO_4)_2 \cdot 12H_2O$) in distilled water slightly acidified with 1 M hydrochloric acid and making up to one litre.

2.1.2 Dilute standard iron solution containing 100 mg iron per litre.2.2. Apparatus

2.2.1 Rotary evaporator with thermostatically controlled waterbath.

2.2.2. Atomic absorption spectrophotometer equipped with an air-acetylene burner.

2.2.3. Iron hollow cathode lamp.2.3. Procedure

2.3.1.Preparation of sample

Remove the alcohol from the wine by reducing the volume of the sample to half its original volume using a rotary evaporator (50 to 60 °C). Make up to the original volume with distilled water.

If necessary, dilute prior to the determination.2.3.2. Calibration

Place 1, 2, 3, 4 and 5 ml of the solution containing 100 mg iron per litre (2.1.2) respectively into a set of 100 ml volumetric flasks and make up to 100 ml with distilled water. The solutions prepared in this way contain 1, 2, 3, 4 and 5 mg of iron per litre respectively.

These solutions should be kept in polyethylene bottles.2.3.3. Determination

Set the wavelength to 248,3 nm. Zero the absorbence scale using distilled water. Aspirate the diluted sample directly into the burner of the spectrophotometer, followed in succession by the five standard solutions (2.3.2). Read off the absorbences. Repeat each measurement.2.4. Expression of results

2.4.1. Method of calculation

Plot a graph giving the variation in absorbence as a function of the iron concentration in the standard solutions. Record the mean value of the absorbence obtained with the diluted wine sample on this this graph and determine its iron concentration C.

The iron concentration in milligrams per litre of the wine to one decimal place will be F.C, where F is the dilution factor.3. USUAL METHOD

3.1. Reagents

3.1.1. Hydrogen peroxide, H_2O_2, 30 % (m/v) solution, iron-free.

3.1.2. 1 M hydrochloric acid, iron free.

3.1.3. Ammonium hydroxide (r20 = 0,92 g/ml).

3.1.4. Pumice stone grains, treated with boiling hydrochloric acid 1:2 dilution and washed with distilled water.

3.1.5 Hydroquinone solution, $C_6H_6O_2$, 2,5 %, acidified with 1 ml concentrated sulphuric acid (r20 = 1,84 mg/l) per 100 ml of solution. This solution must be kept in a yellow bottle in the refrigerator and discarded at the slightest sign of darkening.

3.1.6. Sodium sulphite solution, Na_2SO_3, 20 %, prepared from neutral anhydrous sodium sulphite.

3.1.7. Orthophenanthroline solution, $C_{12}H_8N_2$, 0,5 % in 96 % vol alcohol.

3.1.8. Ammonium acetate solution, CH_3COONH_4, 20 % (m/v).

3.1.9. Fe(III) solution with 1 g of iron per litre. Use of a commercial solution is preferred. Alternatively, a 1 000 mg/l Fe(III) solution can be prepared by dissolving 8,6341 g of ferric ammonium sulphate, $(FeNH_4(SO_4)_2 \cdot 12H_2O)$, in 100 ml of 1 M hydrochloric acid (3.1.2) and making up the volume to one litre with the 1 M hydrochloric acid (3.1.2).

3.1.10. Dilute standard iron solution containing 100 milligrams of iron per litre.3.2. Apparatus

3.2.1. Kjeldahl flask, 100 ml.

3.2.2. Spectrophotometer enabling measurements to be made at a wavelength of 508 nm.3.3. Procedure

3.3.1. Digestion

3.3.1.1. For wines with sugar content below 50 g/l:

Introduce 25 ml of the wine, 10 ml of the hydrogen peroxide solution (3.1.1) and a few grains of pumice (3.1.4) into the 100 ml Kjeldahl flask. Concentrate the mixture to a volume of 2 to 3 ml.

Allow to cool and add to the residue, taking care not to wet the walls of the flask, sufficient ammonium hydroxide (3.1.3) to make the liquid alkaline and precipitate any hydroxides.

After cooling, add 1 M hydrochloric acid (3.1.2) to the alkaline liquid in sufficient quantity to dissolve the precipitated hydroxides and transfer the solution obtained to a 100-ml volumetric flask. Rinse the Kjeldahl flask with 1 M hydrochloric acid (3.1.2) and add the rinsings to the volumetric flask to make the volume up to 100 ml.3.3.1.2. For musts and wines with sugar content above 50 g/l:

3.3.1.2.1. If the sugar content is between 50 and 200 mg/l, the 25 ml wine sample is treated with 20 ml of hydrogen peroxide solution (3.1.1).

Proceed as in 3.3.1.1.

3.3.1.2.2. If the sugar content is above 200 g/l, the samples of wine or must should be diluted 1:2 or possibly 1:4 before being treated as in 3.3.1.2.1.3.3.2. Blank experiment

Carry out a blank experiment with distilled water, using the same volume of hydrogen peroxide solution (3.1.1) as that used for digestion and following the same procedure as that described in 3.3.1.1.3.3.3. Determination

Introduce 20 ml of the hydrochloric acid solution produced by the digestion procedure (3.3.1.1) and 20 ml of the hydrochloric acid solution from the 'blank experiment' (3.3.2) into two separate 50 ml volumetric flasks. Add 2 ml of hydroquinone solution (3.1.5), 2 ml of sulphite solution (3.1.6) and 1 ml of orthophenanthroline solution (3.1.7) to each flask. Allow to stand for 15 minutes, during which time the Fe(III) is reduced to Fe(II). Add 10 ml of ammonium acetate solution (3.1.8), make up to 50 ml with distilled water and shake the two volumetric flasks. Use the solution originating from the blank experiment to set the zero on the absorbence scale at 508

nm and measure the absorbence of the solution to be determined at the same wavelength.3.3.4. Calibration

Place 0,5, 1, 1,5 and 2 ml of the 100 mg of iron per litre solution (3.1.10) into a set of 50 ml volumetric flasks and add 20 ml of distilled water to each. Carry out the procedure described in 3.3.3 to measure the absorbence of each of these prepared standard solutions, which contain 50, 100, 150 and 200 micrograms of iron respectively.3.4. Expression of results

3.4.1. Method of calculation

Plot a graph giving the variations in absorbence as a function of the iron concentrations in the standard solutions. Record the absorbence obtained with the solution to be examined and calculate the iron concentration C in the 20 ml hydrochloric acid sample produced by the digestion procedure, i.e. in 5 ml of the wine sample to be analysed.

The iron concentration in milligrams per litre of the wine to one decimal place will be 200 C.

If the wine (or must) has been diluted, the iron concentration in milligrams per litre of the wine to one decimal place will be 200 × F × C, where F is the dilution factor.

31. COPPER 1. PRINCIPLE OF THE METHOD

The method is based on the use of atomic absorption spectrophotometry.2. APPARATUS

2.1. Platinum dish.

2.2. Atomic absorption spectrophotometer.

2.3. Copper hollow cathode lamp.

2.4. Gas supplies: air-acetylene or nitrous oxide/acetylene.3. REAGENTS

3.1. Metallic copper.

3.2. Nitric acid, HNO3, concentrated 65 %, r20 = 1,38 g/ml.

3.3. Dilute nitric acid, 1:2 (v/v).

3.4. Solution containing copper at 1 g/l.

Use a standard commercial copper solution. This solution may be prepared by weighing 1,000 g of metallic copper and transferring it without loss to a 1 000 ml volumetric flask. Add 1:2 (v/v) dilute nitric acid (3.3) in just sufficient quantity to dissolve the metal, add 10 ml of concentrated nitric acid (3.2) and make up to the mark with doubly distilled water.3.5. Solution containing copper at 100 mg/l.

Introduce 10 ml of the solution prepared as in 3.4 into a 100 ml volumetric flask and make up to the mark with doubly distilled water.4. PROCEDURE

4.1. Preparation of sample and determination of copper

If required, prepare a suitably dilute solution with doubly distilled water.4.2. Calibration

Pipette 0,5, 1 and 2 ml of solution 3.5 (100 mg of copper per litre) into 100 ml volumetric flasks and make up to the volume with doubly distilled water: the solutions so obtained contain 0,5, 1 and 2 mg of copper per litre respectively.

4.3. Measure the absorbence at 324,8 nm. Set the zero with doubly distilled water. Measure directly the absorbence of successive standard solutions prepared in 4.2, Carry out in duplicate.
5. EXPRESSION OF RESULTS

5.1. Method of calculation

Plot a graph giving the variations in absorbence as a function of the copper concentrations in the standard solutions.

Using the measured absorbence of the samples, read off the concentration C in mg/l from the calibration curve.

If F is the dilution factor, the concentration of the copper present is given in milligrams per litre by $F \times C$. It is quoted to two decimal places.

Notes:(a) Select the solutions for establishing the calibration curve and the dilutions of the sample appropriate to the sensitivity of the apparatus to be used and the concentration of the copper present in the sample.(b) Proceed as follows when very low copper concentrations are expected in the sample to be analysed. Place 100 ml of the sample in a platinum dish and evaporate on a waterbath at 100 °C until it becomes syrupy. Add 2,5 ml of concentrated nitric acid (3.2) drop by drop, covering the bottom of the dish completely. Carefully ash the residue on an electric hotplate or over a low flame; then place the dish in a muffle furnace set at 500 ± 25 °C and leave for about one hour. After cooling, moisten the ash with 1 ml of concentrated nitric acid (3.2) while crushing it with a glass rod; allow the mixture to evaporate and ash again as before. Place the dish in the muffle furnace again for 15 minutes; repeat the treatment with nitric acid at least three times. Dissolve the ash by adding 1 ml of concentrated nitric acid (3.2) and 2 ml of doubly distilled water to the dish and transfer to a 10 ml flask. Wash the dish three times using 2 ml of doubly distilled water each time. Finally, make up to the volume with doubly distilled water.

32. CADMIUM 1. Principle

The cadmium is determined directly in the wine by non-flame atomic absorption spectrophotometry.2. APPARATUS

All the glassware must be washed prior to use in concentrated nitric acid heated to 70 to 80 °C and rinsed in double distilled water.

2.1. Atomic absorption spectrophotometer equipped with a graphite oven, background correction and a multipotentiometer.

2.2. Cadmium hollow cathode lamp.

2.3. 5 µl micropipettes with special tips for atomic absorption measurements.3. REAGENTS

The water used must be double distilled using borosilicate glass apparatus, or water of a similar purity. All reagents must be of recognized analytical reagent grade and, in particular, free of cadmium.

3.1. 85 % phosphoric acid (r20 = 1,71 g/ml).

3.2. Phosphoric acid solution obtained by diluting 8 ml of phosphoric acid with water to 100 ml.

3.3. A 0,02 M solution of di-sodium salt of ethylene diamine tetra-acetic acid (EDTA).

3.4. pH 9 buffer solution: dissolve 5,4 g of ammonium chloride in a few millilitres of water in a 100 ml volumetric flask, add 35 ml of ammonium hydroxide solution (r20 = 0,92 g/ml) diluted to 25 % (v/v) and make up to 100 ml with water.

3.5. Eriochrome black T: 1 % (w/w) solid solution in sodium chloride.3.6. Cadmium sulphate ($CdSO_4 \cdot 8H_2O$).

The titre of the cadmium sulphate must be verified using the following method:Weigh exactly 102,6 mg of the cadmium sulphate sample into a cylindrical vessel with some water and shake until dissolved; add 5 ml of the pH 9 buffer solution and approximately 20 mg of eriochrome black T. Titrate with the EDTA solution until the indicator begins to turn blue.

The volume of EDTA added must be equal to 20 ml. If the volume is slightly different, correct the weighed test portion of cadmium sulphate used in the preparation of the reference solution accordingly.3.7. Cadmium reference solution at 1 g per litre.

Use a standard commercial grade solution. This solution may be obtained by dissolving 2,2820 g of cadmium sulphate in water and making up to one litre. Keep the solution in a borosilicate glass bottle with a ground glass stopper.4. PROCEDURE

4.1. Preparation of the sample

Dilute the wine to 1:2 (v/v) with the phosphoric acid solution.
4.2. Preparation of the calibration range of solutions

Using the cadmium reference solution, prepare successive dilutions with titres of 2,5, 5, 10 and 15 µg of cadmium per litre respectively.4.3. Determination

4.3.1. Programming of oven (for guidance only):

Drying at 100 °C for 30 secondsMineralization at 900 °C for 20 secondsAtomization at 2 250 °C for 2 to 3 secondsNitrogen flow (flushing gas): 6 litres/minute

Note: At the end of the procedure, increase the temperature to 2 700 °C to clean the oven.4.3.2. Atomic absorption measurements:

Select wavelength 228,8 nm. Set the zero on the absorbence scale with double distilled water. Using a micropipette, introduce into the oven three 5-µl portions of each of the solutions in the calibration range and of the sample solution to be analysed. Record the absorbences measured.

Calculate the mean absorbence value from the results for the three portions.5. EXPRESSION OF RESULTS

5.1. Method of calculation

Draw the absorbence variation curve as a function of the concentrations of cadmium in the solutions in the calibration range. Variation is linear. Enter the mean absorbence value of the sample solution on the calibration curve, derive from it the cadmium concentration C. The cadmium concentration expressed in micrograms per litre of wine is equal to:2 C

33. SILVER 1. PRINCIPLE OF THE METHOD

The method is based on the use of atomic absorption spectrophotometry after ashing of the sample.2. APPARATUS

2.1. Platinum dish.

2.2. Waterbath, thermostatically controlled to 100 C.

2.3. Furnace controlled to 500 to 525 C.

2.4. Atomic absorption spectrophotometer.

2.5. Silver hollow cathode lamp.

2.6. Gas supplies: air, acetylene.3. REAGENTS

3.1. Silver nitrate, $AgNO_3$.

3.2. Nitric acid, HNO_3, concentrated 65 %, r_{20} = 1,38 g/ml.

3.3. Dilute nitric acid, 1:10 (v/v).3.4. Solution containing silver at 1 g/l.

Use a standard commercial silver solution.This solution may be prepared by dissolving 1,575 g of silver nitrate in dilute nitric acid and making up to a volume of 1 000 ml with dilute nitric acid (3.3).3.5. Solution containing silver at 10 mg/l.

10 ml of the solution prepared as in 3.4 are diluted to 1 000 ml with dilute nitric acid.4. PROCEDURE

4.1. Preparation of sample

Place 20 ml of the sample in a platinum dish and evaporate to dryness over a boiling waterbath. Ash in the furnace at 500 to 525 C. Moisten the white ash with 1 ml of concentrated nitric acid (3.2). Evaporate over a boiling waterbath, repeat the addition of 1 ml nitric acid (3.2) and evaporate a second time. Add 5 ml of dilute nitric acid (3.3) and heat slightly until dissolved.4.2. Calibration

Pipette 2, 4, 6, 8, 10 and 20 ml of solution 3.5 (10 mg of silver per litre) respectively into a set of 100 ml volumetric flasks and make up to the mark with dilute nitric acid (3.3): the solutions so

obtained contain 0,20, 0,40, 0,60, 0,80, 1,0 and 2,0 mg of silver per litre respectively.4.3. Set the wavelength to 328,1 nm. Set zero using doubly distilled water. Measure the absorbence directly of successive standard solutions prepared in 4.2. Carry out in duplicate.
5. EXPRESSION OF RESULTS

5.1. Method of calculation

Plot a graph giving the variations in absorbence as a function of the silver concentrations in the standard solutions.

Using the measured absorbence of the sample, read off the concentration C in mg/l from the calibration curve.

The concentration of silver in the wine is given in milligrams per litre by 0,25C. It is quoted to two decimal places.

Note: Select the concentration of the solutions for the preparation of the calibration curve, the volume of the sample taken and the final volume of the liquid to be appropriate to the sensitivity of the apparatus to be used.5.1. Method of calculation

Plot a graph giving the variations in absorbence as a function of the silver concentrations in the standard solutions.

34. ZINC 1. PRINCIPLE OF THE METHOD

After removal of alcohol, zinc is determined directly in the wine by atomic absorption spectrophotometry.

2. REAGENTS

The water used in borosilicate glass apparatus must be doubly distilled or of an equivalent degree of purity.

2.1. Standard solution containing 1 g of zinc per litre:

use a commercial standard zinc solution. This solution may be prepared by dissolving 4,3975 g of zinc sulphate. ($ZnSO_4 \times 7H_2O$) in water and making up the volume to one litre.

2.2. Dilute standard solution containing 100 mg of zinc per litre.3. APPARATUS

3.1. Rotary evaporator with thermostatically controlled waterbath.

3.2. Atomic absorption spectrophotometer equipped with an air-acetylene burner.

3.3. Zinc hollow cathode lamp.4. PROCEDURE

4.1. Preparation of sample

Remove the alcohol from 100 ml of the wine by reducing the volume of the sample to half its original volume using a rotary evaporator (50 to 60 C). Make up to the original volume of 100 ml with doubly distilled water.4.2. Calibration

Place 0,5, 1, 1,5 and 2 ml of the solution containing 100 mg zinc per litre (2.2) into each one of a set of 100-ml volumetric flasks and make up to the mark with doubly distilled water. The solutions prepared in this way contain 0,5, 1, 1,5 and 2 mg of zinc per litre respectively.4.3. Determination

Set the wavelength to 213,9 nm. Zero the absorbence scale using doubly distilled water. Aspirate the wine directly into the burner of the spectrophotometer, followed in succession by the four standard solutions. Read the absorbences. Repeat each measurement.5. EXPRESSION OF RESULTS

5.1. Method of calculation

Plot a graph giving the variation in absorbence as a function of zinc concentration in the standard solutions. Record the mean value of the absorbence obtained with the diluted wine sample on this graph and determine its zinc concentration to one decimal place.

35. LEAD 1. PRINCIPLE

The lead is determined directly in the wine by non-flame atomic absorption spectrophotometry.2. APPARATUS

All the glassware must be washed prior to use in concentrated nitric acid heated to 70 to 80 C and rinsed in double distilled water.

2.1. Atomic absorption spectrophotometer equipped with a graphite oven, non-specific absorption correction and a multipotentiometer.

2.2. Lead hollow cathode lamp.

2.3. 5 µl micropipettes with special tips for atomic absorption measurements.3. REAGENTS

All reagents must be of recognized analytical reagent grade, and in particular, free of lead. The water used must be doubly distilled using borosilicate glass apparatus, or water of a similar purity.

3.1. 85 % phosphoric acid (r20 = 1,71 g/ml).

3.2. Phosphoric acid solution obtained by diluting 8 ml of phosphoric acid with water to 100 ml.

3.3. Nitric acid (r20 = 1,38 g/ml).

3.4. Lead solution at 1 g per litre.

Use a standard commercial grade solution. This solution may be obtained by dissolving 1,600 g of lead (II) nitrate, Pb(NO3)2 in nitric acid diluted to 1 % (v/v) and made up to one litre. Keep the solution in a borosilicate glass bottle with a ground glass stopper.4. PROCEDURE

4.1. Preparation of the sample

Dilute the wine to 1:2 or 1:3 with the phosporic acid solution, depending on the presumed lead concentration.4.2. Preparation of the calibration range of solutions

Using the lead reference solution, prepare successive solutions with titres of 2,5, 5, 10 and 15 µg of lead per litre respectively, by diluting with double distilled water.4.3. Determination

4.3.1 Programming of oven (for guidance only):

Drying at 100 C for 30 secondsMineralization at 900 C for 20 seconds
Atomization at 2 250 C for 2 to 3 secondsNitrogen flow (flushing gas): 6 litres/minute.

Note: At the end of the procedure, increase the temperature to 2 700 C to cleanse the oven.4.3.2. Measurements

Select wavelength 217 nm. Set the zero on the absorbence scale with doubly distilled water. Using a micropipette, introduce into the programmed oven three 5-µl portions of each of the solutions in the calibration range and of the sample solution to be analysed. Record the absorbences measured. Calculate the mean absorbence value from the results for the three portions.5. EXPRESSION OF RESULTS

5.1. Method of calculation

Draw the absorbence variation curve as a function of the concentrations of lead in the calibration range. The variation is linear. Carry over the mean absorbence value of the sample solution on the calibration curve, derive from it the lead concentration C. The lead concentration expressed in micrograms per litre of wine is equal to:C × FWhere F = the dilution factor.

36. FLUORIDES 1. PRINCIPLE

The fluoride content of the wine, added to a buffer solution, is determined using a solid membrane selective electrode. The measured potential is proportionate to the logarithm of the activity of the fluoride ions in the medium being analysed, in accordance with the following equation:E = Eo ± S log aF (1)WhereE = potential of the ion-selective electrode measured against the reference electrode in the medium being analysed; Eo = standard potential of the sensor; S = slope of the ion-selective electrode (Nernst factor). At 25 C the theoretical slope is equal to 59,2 mV; aF = activity of the fluoride ions in the solution being analysed.2. APPARATUS

2.1. Fluoride-ion-selective crystal membrane electrode.

2.2. Reference electrode (calomel or Ag/AgCl).

2.3. Millivoltmeter (pH meter with extended scale in millivolts), accurate to 0,1 mV.

2.4. Magnetic stirrer with an insulating plate to protect the analysis solution from the heat of the motor. Stirring vessel covered with plastic (polythene or equivalent material).

2.5. Plastic beakers with a capacity of 30 or 50 ml, and plastic bottles (polythene or equivalent material).

2.6. Precision pipettes (pipettes graduated in microlitres or any other equivalent pipettes).3. REAGENTS

3.1. Stock fluoride solution of 1 g/l.

Use standard commercial quality solution of 1 g/l. This solution can be prepared by dissolving 2,210 g of sodium fluoride (dried for three to four hours at 105 C) in distilled water. Make up to one litre with distilled water. The solution is kept in a plastic bottle.

3.2. Standard fluoride solutions of appropriate concentration are prepared by diluting the stock solution with distilled water and kept in plastic bottles. Solutions the fluoride content of which is in mg/l must not be prepared in advance.

3.3. Buffer solution, pH 5,5

10 g of trans-1,2-dicmiaocyclohexane tetra-acetic acid (CDTA) are added to water (about 50 ml); add a solution containing 58 g of sodium chloride and 29,4 g of trisodium citrate in 700 ml of distilled water. The CDTA is dissolved by adding approximately 6 ml of 32 % (m/v) sodium hydroxide solution.

Lastly, add 57 ml of acetic acid (r20 = 1,05 g/ml) and bring the pH to 5,5 with 32 % sodium hydroxide solution (about 45 ml). Leave to cool and make up to one litre with distilled water.
4. PROCEDURE

Preliminary comment:Care should be taken to ensure that all the solutions remain at a temperature of 25 °C (± 1 °C) during measurement. (A deviation of more than 1 C causes a modification of about 0,2 mV.)4.1. Direct method

Place a defined volume of wine in a plastic beaker with an equal volume of buffer solution.

The solution is stirred in an even and moderate manner. When the indicator is stable (stability is reached when the potential varies by not more than 0,2 to 0,3 mV/three minutes), read the value of the potential in mV.4.2.The known additions method

Stirring continuously, add a known volume of standard fluoride solution to the analysis medium using a precision pipette. When the indicator is stable, read the value of the potential in mV.

The concentration of the standard solution to be added is selected as follows:(a) double or treble the fluoride concentration in the analysis medium; (b) the volume of the analysis medium must remain practically constant (an increase in volume of 1 % or less).(Condition (b) simplifies the calculations, see 5.)

The approximate concentration of the analysis medium is read on a calibration line drawn on a semi-logarithmic scale with the standard fluoride solutions with titres of 0,1, 0,2, 0,5, 1,0, 2,0 mg/l.

Note: If the approximate concentration of the analysis medium lies outside the concentration range of the standard solutions, dilute the sample.

Example:

If the approximate fluoride content of the analysis medium (20 ml volume) is 0,25 mg/l F ; the concentration must be increased by 0,25 mg/l. To do this, use the appropriate delivery pipette to add, for example, 0,20 ml (= 1 %) of a standard solution containing 25 mg/l F or 0,050 ml of a standard solution with 100 mg/l F .5. CALCULATIONS

The fluoride content of the analysis medium expressed in mg/l is obtained by applying the following formula:CF = fluoride concentration of the analysis medium (mg/l); Ca = concentration of fluoride added (mg/l) to analysis medium (Va); Vo = initial volume of the analysis medium before overloading (ml); Va = volume of the overloaded solution (ml); DE = difference between potentials E1 and E2 obtained in 4.1 and 4.2 (mV); S = slope of the electrode in the analysis solution.

If Va is very close to Vo (see 4.2), the following simplified formula is applied:
The value obtained must be multiplied by the dilution factor arising from addition of the buffer solution.

37. CARBON DIOXIDE 1. PRINCIPLE OF METHODS

1.1. Reference method

1.1.1. Still wines(CO2 over pressure E 0,5 × 105 Pa) (11)

The volume of wine taken from the sample is cooled to around 0 °C and mixed with a sufficient quantity of sodium hydroxide to give a pH of 10 to 11. Titration is carried out with an acid solution in the presence of carbonic anhydrase. The carbon dioxide content is calculated from the volume of acid needed to change the pH from 8,6 (bicarbonate form) to 4,0 (carbonic acid). A blank titration is carried out in the same conditions on decarbonated wine in order to take account of the volume of sodium hydroxide solution taken up by the wine acids.1.1.2. Sparkling and semi-sparkling wines

The sample of wine to be analysed is cooled near to freezing point. After removal of a quantity to be used as a blank after decarbonation, the remainder of the bottle is made alkaline to fix all the carbon dioxide in the form of Na2CO3. Titration is carried out with an acid solution in the presence of carbonic anhydrase. The carbon dioxide content is calculated from the volume of acid solution needed to change the pH from 8,6 (bicarbonate form) to 4,0 (carbonic acid). A blank titration is carried out in the same conditions in decarbonated wine in order to take account of the volume of sodium hydroxide taken up by the wine acids.1.2. Usual method: sparkling and semi-sparkling wines

Manometric method: the excess pressure of the carbon dioxide is measured directly in the bottle using an aphrometer.2. REFERENCE METHOD

2.1. Still wines (CO2 over pressure d 0,5 × 105 Pa)2.1.1. Apparatus

2.1.1.1. Magnetic stirrer.

2.1.1.2. pH meter.2.1.2. Reagents

2.1.2.1. Sodium hydroxide solution, NaOH, 0,1 M.

2.1.2.2. Sulphuric acid solution, H2SO4, 0,05 M.

2.1.2.3. Carbonic anhydrase solution, 1 g/l.2.1.3. Procedure

Cool the wine sample to approximately 0 °C together with the 10 ml pipette used for sampling.

Place 25 ml of sodium hydroxide solution (2.1.2.1) in a 100 ml beaker; add two drops of aqueous solution of carbonic anhydrase (2.1.2.3). Introduce 10 ml of wine using the pipette cooled to 0 °C.

Place the beaker on the magnetic stirrer, set up the pH electrode and stir moderately.

When the liquid has reached room temperature, titrate slowly with the sulphuric acid solution (2.1.2.2) until the pH reaches 8,6. Note the burette reading.

Continue titrating with the sulphuric acid (2.1.2.2) until the pH reaches 4,0. Let nml be the volume used between pH 8,6 and 4,0.

Remove CO2 from approximately 50 ml of the wine sample by agitation under vacuum for three minutes, the flask being heated in a waterbath to about 25 °C.

Carry out the above procedure on 10 ml of the decarbonated wine. Let n2 ml be the volume used.2.1.4. Expression of results

1 ml of the titrated 0,1 M sodium hydroxide solution corresponds to 4,4 mg of CO2.

The quantity of CO2 in grams per litre of wine is given by the formula:0,44 (n - n2)It is quoted to two decimal places.

Note: Where wines contain little CO2 (CO2 < 1 g/l), the addition of carbonic anhydrase to catalyse the hydration of CO2 is unnecessary.2.2. Sparkling and semi-sparkling wines

2.2.1. Apparatus

2.2.1.1. Magnetic stirrer.

2.2.1.2. pH meter.2.2.2. Reagents

2.2.2.1. Sodium hydroxide, NaOH, 50 % (m/m).

2.2.2.2. Sulphuric acid solution, H2SO4, 0,05 M.

2.2.2.3. Carbonic anhydrase solution, 1 g/l.2.2.3.Procedure

Mark the level of wine in the bottle and then cool until freezing begins. Allow the bottle to warm up slightly, while shaking, until ice crystals disappear. Remove the stopper rapidly and place 45 to 50 ml of wine in a measuring cylinder for blank titration. The exact volume removed, v ml, is determined by reading on the cylinder after it has returned to room temperature.

Immediately after the blank sample has been removed, add 20 ml of the sodium hydroxide solution (2.2.2.1) in the bottle with a capacity of 750 ml.

Wait until the wine has reached room temperature.

Place 30 ml of boiled distilled water and two drops of the carbonic anhydrase solution (2.2.2.3) into a 100 ml beaker. Add 10 ml of wine which has been made alkaline. Place the beaker on the magnetic stirrer, set up the electrode and magnetic rod and stir moderately.

Titrate with the sulphuric acid solution (2.2.2.2) slowly until the pH reaches 8,6. Note the burette reading.

Continue titrating slowly with the sulphuric acid (2.2.2.2) until the pH reaches 4,0. Let n ml be the volume used between pH 8,6 and 4,0.

Remove CO2 from the v ml of wine placed on one side for the blank titration by agitating under vacuum for three minutes, the flask being heated in a waterbath at about 25 °C. Remove 10 ml of decarbonated wine and add to 30 ml of boiled distilled water, add two to three drops of sodium hydroxide solution (2.2.2.1) to bring the pH to 10 to 11. Then follow the above procedure. Let n2 ml be the volume of 0,5 M sulphuric acid added.2.2.4. Expression of results

1 ml of 0,05 M sulphuric acid corresponds to 4,4 mg of CO2.

Empty the bottle of wine which has been made alkaline and determine to within 1 ml the initial volume of wine by making up to the mark with water, say V ml.

The quantity of CO2 in grams per litre of wine is given by the following formula:The result is quoted to two decimal places.2.3. Expression of results

The excess pressure at 20 °C (Paph20) expressed in pascals is given by the formula:where:Q = CO2 content in g/l of wine,A = the alcoholic strength of wine at 20 °C,S = the sugar content of the wine in g/l,Patm = the atmospheric pressure, expressed in pascals.3. USUAL METHOD: SPARKLING AND SEMI-SPARKLING WINES

3.1. Apparatus

3.1.1. Aphrometer

The apparatus enabling the excess pressure in bottles of sparkling and semi-sparkling wine is called an aphrometer. It takes a different form according to the way in which the bottle is stoppered (metallic top, cap, cork or plastic stopper, see Figures 1 and 2).

They are graduated in pascals (Pa) (12), although it is more practical to use 105 Pa or the kilopascal (kPa) as the unit.

They fall into various classes. The class of a manometer is the precision of a reading in relation to a full scale reading expressed as a percentage (e.g. a 1 000 kPa manometer class I means that the

maximum pressure of 1 000 kPa can be read to within ±10 kPa). A class I instrument is recommended for accurate measurement.

Aphrometers must be checked regularly (at least once a year).3.2. Procedure

Measurements must be carried out on bottles whose temperature has been stabilized for at least 24 hours.

After having pierced the cap, the cork or the stopper, the bottle must then be shaken vigorously until the pressure is constant in order to take the reading. Figure 1: Aphrometer for capsFigure 2: Aphrometer for corks and stoppers 3.3. Expression of results

The excess pressure at 20 °C (Paph20) is expressed in pascals (Pa) or in kilopascals (kPa).

It must be quoted in a form consistent with the precision of the manometer (e.g. 6,3 × 105 Pa or 630 kPa and not 6,33 × 105 Pa or 633 kPa for a class I manometer with a full scale reading of 1 000 kPa).

If the temperature at which the mesurement is carried out is different from 20 °C, a correction should be made by multiplying the measured pressure by the coefficientgiven in Table I. This relates the result to 20 °C.4. RELATIONSHIP BETWEEN THE PRESSURE AND THE QUANTITY OF CARBON DIOXIDE CONTAINED IN A SEMI-SPARKLING WINE (13)

From the excess pressure at 20 °C (Paph20), the absolute pressure at 20 °C (Pabs20) is calculated using the formula:Pabs20 = Patm + Paph20

where Patm is the atmospheric pressure expressed in bars.

The quantity of carbon dioxide contained in a wine is given by the following relationships:- in litres of CO2 per litre of wine:0,987 × 10-5 Pabs20 (0,86 0,01A) (1 0,00144S),- in grams of CO2 per litre of wine:1,951 × 10-5 Pabs20 (0,86 0,01A) (1 0,00144S),whereA is the alcoholic strength of the wine at 20 °C,S is the sugar content of the wine in grams per litre.
Table IRatio of the excess pressure Paph20 in a sparkling or semi-sparkling wine at 20 °C to the excess pressure Papht at a temperature

38. CYANIDE DERIVATIVES 1. PRINCIPLE OF THE METHODS

1.1. Rapid test method

Testing for wines treated with potassium hexacyanoferrate (II).

Testing for the absence of iron (III) hexacyanoferrate (II) in suspension in the deposit.

Testing for the absence of the formation of iron (III) hexacyanoferrate (II) by the addition of an iron (III) salt to the acidified wine.

Testing for the presence of iron precipitated by the addition to the acidified wine of a mixture of alkali hexacyanoferrate (II) and hexacyanoferrate (III).1.2. Usual method

Argentometric determination of the total hydrocyanic acid liberated by acid hydrolysis and separated by distillation.2. RAPID TEST METHOD

Checking of wines treated with potassium hexacyanoferrate (II).2.1. Apparatus

One of the following pieces of equipment should be available:

2.1.1. Centrifuge producing centrifugal forces from 1 200 to 1 500 g.

2.1.2. Filtering apparatus with membrane filters (pore diameter, 0,45 µm).2.2.Reagents

2.2.1. Hydrochloric acid, diluted 1:2 (v/v) obtained by diluting iron-free hydrochloric acid, HC/l (r20 = 1,18 to 1,19 g/ml).

2.2.2 Iron (III) ammonium sulphate, (Fe2(SO4)3, (NH4)2SO4, 24H2O,) 15 % (m/v) solution.

2.2.3. Potassium hexacyanoferrate (II), (K4 [FE(CN)6], 3H2O) 10 % (m/v)

2.2.3. Potassium hexacyanoferrate (III), (K3 [FE(CN)6]) 10 % (m/v) solution. To be prepared just before use.2.3. Procedure

2.3.1. Test for traces of iron (III) hexacyanoferrate (II) in suspension

After shaking the sample, place 20 ml of wine in a 30 ml conical centrifuge tube. Add 1 ml dilute hydrochloric acid (2.2.1). Centrifuge for 15 minutes or filter through a membrane filter of pore diameter 0,45 µm. The deposit obtained after centrifuging or the filter should be completely free of blue particles.2.3.2. Test for traces of hexacyanoferrate (II) ions in solution

Add to the supernatant liquid or the filtrate from test 2.3.1 one drop of iron (III) ammonium sulphate solution (2.2.2). Stir and allow to stand for at least 24 hours.
Centrifuge for 15 minutes or filter through a membrane filter of pore diamter 0,45 µm. The deposit after centrifuging or the filter should be completely free of blue particles of iron (III) hexacynoferrate (II).2.3.3. Test for the presence of iron ions in the wine

Place 20 ml of wine, 1 ml of hydrochloric acid (2.2.1), one drop of potassium hexacyanoferrate (II) solution (2.2.3) and one drop of potassium hexacyanoferrate (III) solution (2.2.4) in a test tube. A blue coloration or a blue precipitate should appear in less than 30 minutes. After centrifuging or after filtering through a membrane filter of pore diameter 0,45 µm, followed by rinsing twice with 5 ml of water, a blue deposit should be observed in the centrifuge tube or on the membrane filter.3. USUAL METHOD

3.1. Apparatus

3.1.1. Distillation apparatus, consisting of a 500-ml round-bottomed flask joined by a tube with ground glass joints to the upper end of a vertically held condenser at least 350 mm long.

The lower end of the condenser is attached to an adapter with a drawn-out portion to conduct the distillate to the bottom of a 50-ml flask immersed completely in iced water.3.1.2. Boiling electric water bath (thermostatically controlled)

3.2. Reagents

3.2.1. Sulphuric acid, diluted 1:5 (v/v).

Add 200 ml of sulphuric acid, H_2SO_4, (r_{20} = 1,84 g/ml) with great care to sufficient water to produce one litre of solution,

3.2.2. Crystalline copper (II) chloride, $CuCl_2 \cdot 2H_2O$.

3.2.3. Phenol red solution

Dissolve 0,05 g of phenol red in 1,4 ml of a 0,1 M sodium hydroxide solution; make up the solution to 1 000 ml.

3.2.4. Potassium iodide solution.

Dissolve 250 g potassium iodide, KI, in sufficient water to obtain one litre of solution.

3.2.5. Silver nitrate solution 0,001 M

Add 0,5 ml of concentrated nitric acid, HNO_3 (r_{20} = 1,40 g/ml) to 10 ml of 0,1 M silver nitrate solution, $AgNO_3$ and make up to one litre with water.

3.2.6. Sodium hydroxide solution, 1 M, iron-free.3.3. Procedure

Introduce 100 ml of filtered wine (or in the unfiltered state if the determination of hydrocyanic acid contained in any blue turbidity is also required), add approximately 5 mg of copper (II) chloride (3.2.2) and 10 ml of dilute sulphuric acid (3.2.1). Place 5 ml of sodium hydroxide solution (3.2.6) into the receiver flask. Distil until the 50-ml flask is full.

Transfer the distillate to a 400-ml beaker and place on a boiling waterbath; accelerate the evaporation by directing a fairly strong current of cold air produced by a blower across the surface of the alkaline liquid. The volume should be reduced to 5 to 7 ml, which will take about 30 minutes (ensure that the volume is never reduced to less than 5 ml).

Filter the cooled solution if necessary, collecting the filtrate in a cylindrical tube of 20 mm diameter and 180 mm length, or transfer the solution directly to this tube. Wash the beaker and possibly the filter with a few ml of water and add them to the tube.

Place the glass tube on a black base and allow a beam of white light to fall on it from the side. The liquid should be perfectly clear (14).

Add two drops of phenol red solution (3.2.3) to aid observation of the end point (15) and one drop of potassium iodide solution (3.2.4). Titrate with 0,001 M silver nitrate solution (3.2.5) until a slight but persistent turbidity is observed. Let n be the volume of titrant used to obtain this result.

In addition, prepare a similar tube for a blank test containing 5 ml of sodium hydroxide solution (3.2.6), two drops of phenol red solution (3.2.3)(15), one drop of potassium iodide solution (3.2.4) and sufficient water to obtain an identical volume to that above. Add sufficient silver nitrate solution (3.2.5) to obtain the same turbidity as above. Let the volume used be n2 (16).3.4. Expression of results

1 ml of the 0,001 M silver nitrate solution corresponds to 54 µg of hydrocyanic acid, HCN.

The total hydrocyanic acid contained in one litre of wine is therefore 0,54 (n - n2) mg. The result is quoted to two decimal places.

Regard as significant only those results where (n - n2) is greater than 0,5 ml.

Ifn - n' is greater than 10 ml, repeat the procedure using a 0,01 M silver nitrate solution.

39. ALLYL ISOTHIOCYANATE 1. PRINCIPLE OF THE METHOD

Any allyl isothiocyanate present in the wine is collected by distillation and identified by gas chromatography.2. REAGENTS

2.1. Ethanol, absolute.

2.2. Standard solution: solution of allyl isothiocyanate in absolute alcohol containing 15 mg of allyl isothiocyanate per litre.

2.3. Freezing mixture consisting of ethanol and dry ice (temperature 60 °C).3. APPARATUS

3.1. Distillation apparatus as shown in the figure overleaf. A stream of nitrogen is passed continuously through the apparatus.

3.2. Heating mantle, thermostatically controlled.

3.3. Flowmeter.

3.4. Gas chromatograph fitted with a flame spectrophotometer detector equipped with a selective filter for sulphur compounds (wavelength = 394 nm) or any other suitable detector.

3.5. Stainless steel chromatograph column of internal diameter 3 mm and length 3 m filled with Carbowax 20M at 10 % on Chromosorb WHP, 80 to 100 mesh.

3.6. Microsyringe, 10µl.4. PROCEDURE

Put two litres of wine into the distillation flask, introduce a few millilitres of ethanol (2.1) into the two collecting tubes so that the porous parts of the gas dispersion rods are completely immersed. Cool the two tubes externally with the freezing mixture. Connect the flask to the collecting tubes and begin to flush the apparatus with nitrogen at a rate of three litres per hour. Heat the wine to 80 °C with the heating mantle, distil and collect 45 to 50 ml of the distillate.

Stabilize the chromatograph. It is recommended that the following conditions are used:- injector temperature: 200 °C,- column temperature: 130 °C,- helium carrier gas flow rate: 20 ml per minute.

With the microsyringe, introduce a volume of the standard solution such that the peak corresponding to the allyl isothiocyanate can easily be identified on the gas chromatogram.

Similarly introduce an aliquot of the distillate into the chromatograph. Check that the retention time of the peak obtained corresponds with that of the peak of allyl isothiocyanate.

Under the conditions described above, compounds naturally present in the wine will not produce interfering peaks on the chromatogram of the sample solution.
Apparatus for distillation under a current of nitrogen

40. CHROMATIC PROPERTIES 1. WINES AND MUSTS

1.1. Definitions

The chromatic properties of a wine are defined as its luminosity and its chromaticity.

The luminosity is represented by the transmittance and it varies inversely with the colour intensity of the wine.

Thechromaticity is represented by the dominant wavelength (which characterizes the tint) an the purity of the colour.

By convention, and for reasons of convenience, the chromatic properties of red and rosé wines are given as the colour intensity and the tint, in keeping with a procedure adopted as the usual method.1.2. Principle of the methods

1.2.1. Reference method

This is a spectrophotometric method which makes it possible to determine the tristimulus values and three chromaticity coordinates necessary for the specification of the colour as laid down by the International Commission on Illumination (CIE).1.2.2.Usual method (applicable to red and rosé wines)

This is a spectrophotometric method by which the chromatic properties are expressed by convention as follows:

The colour intensityis given by the sum of the absorbences at wavelengths af 420, 520 and 620 nm for radiation traversing a 1 cm optical path in the sample.

The tint is expressed by the ratio of the absorbences at 420 nm and 520 nm.1.3. Reference method

1.3.1. Apparatus

1.3.1.1. Spectrophotometer enabling measurements to be made between 300 and 700 nm.

1.3.1.2. Glass cells in pairs, with optical paths, b, equal to 0,1, 0,2, 0,5, 1,2 and 4 cm.1.3.2. Procedure

1.3.2.1. Preparation of the sample

Cloudy wine must be clarified by centrifugation. The bulk of the carbon dioxide in young and sparkling wines must be removed by shaking under vacuum.1.3.2.2. Measurements

The optical path, b, in the glass cell should be so chosen that the measured absorbence lies between 0,3 and 0,7.

The following guidance is given for the appropriate choice of the optical path: use cells of 2 (or 4) cm optical path for white wines, with 1 cm for rosé wines and with 0,1 cm (or 0,2 cm) for red wines.

The spectrophotometric measurements should be made using distilled water, in a cell with the same optical path, b, as reference liquid to establish the zero of the absorbence scale at wavelengths 445, 495, 550 and 625 nm.

The four corresponding absorbences for the wine should then be measured to three decimal places for the optical path, b. Let these be A445, A495, A550, A625.1.3.3. Calculations

Together with Table I, use these values of the absorbences for the optical path, b cm to obtain the corresponding transmittances (T %). Let these be T445, T495, T550 and T625.

- Calculate the tristimulus values X, Y and Z expressed as decimal fractions from the following expressions:X = 0,42T625 + 0,35T550 + 0,21T445Y = 0,20T625 + 0,63T550 + 0,17T495Z = 0,24T495 + 0,94T445- Calculate the chromaticity coordinates x and y from:1.3.4. Expression of results

1.3.4.1. The relative luminosity is given by the value of Yexpressed as a percentage. (For complete darkness, Y = 0 %; for colourless liquids, Y = 100 %.)

1.3.4.2. The chromaticity is expressed by the dominant wavelength and the purity.

The determination of these two quantities makes use of the chromaticity diagram bounded by the spectral locus as given in Figure 1. The point O plotted in this diagram represents the white light source used and has the coordinates of a standard source, C,xo = 0,3101 and yo = 0,3163, representing daylight of average brightness.

- Dominant wavelengthPlot the point C with coordinates x, y on the chromaticity diagram.If C is outside the triangle AOB, draw the straight line joining O to C and extend it to cut the spectral locus at the point S, which corresponds to the dominant wavelength.If C is inside the triangle AOB, draw the straight line from C to O and extend it to intersect the spectral locus at a point corresponding to the wavelength of the colour complementary to that of the wine. This wavelength is denoted by its value followed by the letter C.- PurityIf the point C is outside the triangle AOB, the purity is given as a percentage by the ratio:If the point C is inside the triangle AOB, the purity is given as a percentage by the ratio: (17)
where P is the point where the straight line OC cuts the line of purples (line AB).Purity is also given directly by chromaticity diagrams from the known values of x and y (Figures 2, 3, 4, 5 and 6).

1.3.4.3. Results

The colour of a wine is completely defined by its luminosity, its chromaticity (expressed by the dominant wavelength) and its purity.

These should be indicated in the analysis report with the value of the optical path in which the measurements were made.1.4. Usual method

1.4.1. Apparatus

1.4.1.1. Spectrophotometer enabling measurements to be made between 300 and 700 nm.

1.4.1.2. Glass cells in pairs, with optical paths, b, equal to 0,1, 0,2, 0,5 and 1 cm.1.4.2.Preliminary preparation of the sample

Cloudy wine must be clarified by centrifugation.

The bulk of the carbon dioxide in young and sparkling wines must be removed by shaking under vacuum.1.4.3. Procedure

The optical path, b, in the glass cell should be chosen so that the measured absorbence A lies between 0,3 and 0,7.

The spectrophotometric measurements should be made using distilled water, in a cell with the same optical path,b, as reference liquid to establish the zero of the absorbence scale at wavelengths 420, 520 and 620 nm.1.4.4. Expression of results

1.4.4.1. Calculations

Calculate the absorbences at the three wavelengths for an optical path of 1 cm by dividing the measured absorbances by b in cm. Let these be A420, A520 and A620.

The colour intensity I is by convention given by the following expression:I = A420 + A520 + A620It is quoted to three decimal places.

The tint Nis by convention given by the following expression:It is quoted to three decimal places.
TABLE 1

Transformation of absorbences to transmittances (T %)

Method of useFind the first decimal figure of the absorbence in the first vertical column, and call its row R. Find the second decimal figure of the absorbence in the top horizontal row and call its column C. Read the figure in the box at the intersection of the row R and the column C. To calculate the transmittance, divide this figure by 10 if the absorbence is less than 1, by 100 if it lies between 1 and 2, and by 1 000 if it lies between 2 and 3.

Note:The figure in the top right-hand corner of each box enables the third decimal figure of the absorbence to be taken into account by interpolation.

Example:Absorbence 0,47 1,47 2,47 3,47T % 33,9 3,4 0,3 0Transmittances T % are to be expressed to the nearest 0,1 %.

FIGURE 1Chromaticity diagram including all colours in the spectrum

FIGURE 2Chromaticity diagram for unadulterated red (bright red) wines and red-brown (brick red) wines

FIGURE 3Chromaticity diagram for unadulterated red (bright red) wines and red-brown (brick red) wines

FIGURE 4Chromaticity diagram for unadulterated red (bright red) wines and purple wines

FIGURE 5Chromaticity diagram for unadulterated red (bright red) wines and purple wines

FIGURE 6Chromaticity diagram for red-brown (brick red) wines and purple wines

2. RECTIFIED CONCENTRATED MUSTS

2.1. Principle of the method

The absorbence of the rectified concentrated must is measured at 425 nm through a thickness of 1 cm after dilution to bring the sugar concentration to 25 % (m/m) (25° Brix).2.2. Apparatus

2.2.1. Spectrophotometer enabling measurements to be made between 300 and 700 nm.

2.2.2. Glass cells with optical paths of 1 cm.

2.2.3. Membrane filter of pore diameter 0,45 µm.2.3. Procedure

2.3.1.Preparation of the sample

Use the solution with a sugar concentration of 25 % (m/m) (25° Brix) prepared as described in the chapter 'pH', section 4.1.2. Filter is through a membrane filter of pore diameter 0,45 µm.2.3.2. Determination of absorbence

Zero the absorbence scale at a wavelength of 425 nm using a cell with an optical path of 1 cm containing distilled water.

Measure the absorbence A at the same wavelength of the solution containing 25 % sugar (25° Brix) prepared as in 2.3.1 and placed in a cell with an optical path of 1 cm.2.4. Expression of results

The absorbence at 425 nm of the rectified concentrated must in a solution with 25 % sugar (25° Brix) is quoted to two decimal places.

41. FOLIN-CIOCALTEU INDEX 1. DEFINITION

The Folin-Ciocalteu index is the result obtained from the application of the method described below.2. PRINCIPLE OF THE METHOD

All the phenolic compounds contained in the wine are oxidized by the Folin-Ciocalteu reagent. This reagent is formed from a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$) which, after oxidation of the phenols, is reduced to a mixture of the blue oxides of tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}).

The blue coloration produced has a maximum absorption in the region of 750 nm, and it is proportional to the total quantity of phenolic compounds originally present.3. REAGENTS

These must be of analytical reagent quality. The water used must be distilled or water of equivalent purity.3.1. Folin-Ciocalteu reagent

This reagent is available commercially in a form ready for use. It may be prepared as follows: dissolve 100 g of sodium tungstate ($Na_2WO_4 \cdot 2H_2O$) and 25 g of sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) in 700 ml of distilled water. Add 50 ml of 85 % phosphoric acid (r_{20} = 1,71 g/ml) and 100 ml of concentrated hydrochloric acid (r_{20} = 1,19 g/ml). Bring to the boil and boil for 10 hours under reflux conditions. Then add 150 g of lithium sulphate ($Li_2SO_4 \cdot H_2O$) and a few drops of bromine and boil once more for 15 minutes. Allow to cool and make up to one litre with distilled water.

3.2. Anhydrous sodium carbonate, Na_2CO_3, made up into a 20 % m/v solution.4. APPARATUS

Normal laboratory apparatus, particularly:

4.1. 100 ml volumetric flasks.

4.2. Spectrophotometer capable of operating at 750 nm.5. PROCEDURE

5.1. Red wine

Introduce the following into a 100 ml volumetric flask (4.1) strictly in the order given: 1 ml of the wine, previously diluted 1:5,50 ml of distilled water, 5 ml of Folin-Ciocalteu reagent (3.1),20 ml of sodium carbonate solution (3.2).

Make up to 100 ml with distilled water.

Stir to homogenize. Wait 30 minutes for the reaction to stabilize. Determine the absorbence at 750 nm through a path length of 1 cm with respect to a blank prepared with distilled water in place of the wine.

If the absorbence is not around 0,3 an appropriate dilution should be made.
5.2. White wine

Carry out the same procedure with 1 ml of undiluted wine.5.3. Rectified concentrated must

5.3.1. Preparation of sample

Use the solution with a sugar concentration of 25 % (m/m) (25° Brix) prepared as described in the chapter 'pH', section 4.1.2.5.3.2.Measurement

Proceed as described for the case of red wine (5.1) using a 5 ml sample prepared as described in 5.3.1 and measuring the absorbence with respect to a control prepared with 5 ml of a 25 % (m/m) invert sugar solution.6. EXPRESSION OF RESULTS

6.1. Method of calculation

The result is expressed in the form of an index obtained by multiplying the absorbence by 100 for red wines diluted 1:5 (or by the corresponding factor for other dilutions) and by 20 for white wines. For rectified concentrated musts, multiply by 16.6.2. Repeatability

The difference between the results of two determinations carried out simultaneously or very quickly one after the other by the same analyst must not be greater than 1.

Good repeatability of results is achieved by using scrupulously clean apparatus(volumetric flasks and spectrophotometer cells).

42. SPECIAL METHODS OF ANALYSIS FOR RECTIFIED CONCENTRATED GRAPE MUST (a) TOTAL CATIONS

1. PRINCIPLE OF THE METHOD

The test sample is treated by a strongly acid cation exchanger. The cations are exchanged with H+. Total cations are expressed by the difference between the total acidity of the effluent and that of the test sample.2. APPARATUS

2.1. Glass column of internal diameter 10 to 11 mm and length approximately 300 mm, fitted with a drain tap.

2.2. pH meter with a scale graduated at least in 0,1 pH units.

2.3. Electrodes:- glass electrode, kept in distilled water,- calomel/saturated potassium chloride reference electrode, kept in a saturated solution of potassium chloride,- or a combined electrode, kept in distilled water.3. REAGENTS

3.1. Strongly acid cation exchange resin in H+ form pre-swollen by soaking in water overnight.

3.2. Sodium hydroxide solution, 0,1 M.

3.3. Paper pH indicator.4. PROCEDURE

4.1. Preparation of sample

Use the solution obtained by diluting the rectified concentrated must to 40 % (m/v) as described in the chapter 'Total acidity', section 5.1.2.4.2. Preparation of the ion exchange column

Introduce into the column approximately 10 ml pre-swollen ion exchanger in H+ form. Rinse the column with distilled water until all acidity has been removed, using the paper indicator to monitor this.4.3. Ion exchange

Pass 100 ml of the rectified concentrated must solution prepared as in 4.1 through the column at the rate of one drop every second. Collect the effluent in a beaker. Rinse the column with 50 ml of distilled water. Titrate the acidity in the effluent (including the rinse water) with the 0,1 M sodium hydroxide solution until the pH is 7 at 20 °C. The alkaline solution should be added slowly and the solution continuously shaken. Letn ml be the volume of 0,1 M sodium hydroxide solution used.5. EXPRESSION OF RESULTS

The total cations are expressed in milliequivalents per kilogram of total sugar to one decimal place.
5.1. Calculations

- Acidity of the effluent expressed in milliequivalents per kilogram of rectified concentrated must:E = 2,5n- Total acidity of the rectified concentrated must in milliequivalents per kilogram (see 'Total acidity', section 6.1.2): a- Total cations in milliequivalents per kilogram of total sugars:Where P = percentage concentration (m/m) of total sugars.(b) CONDUCTIVITY

1. PRINCIPLE OF THE METHOD

The electrical conductivity of a column of liquid defined by two parallel platinum electrodes at its ends is measured by incorporating it in one arm of a Wheatstone bridge.

The conductivity varies with temperature and it is therefore expressed at 20 °C.2. APPARATUS

2.1. Conductivity meter enabling measurements of conductivity to be made over a range from 1 to 1 000 microsiemens per cm (µS cm-1).

2.2. Waterbath for bringing the temperature of samples to be analysed to approximately 20 °C (20 ± 2 °C).3. REAGENTS

3.1. Demineralized water with specific conductivity below 2 µS cm-1 at 20 °C.

3.2. Reference solution of potassium chloride.

Dissolve 0,581 g of potassium chloride, KCl, previously dried to constant mass at a temperature of 105 °C, in demineralized water (3.1). Make up to one litre with demineralized water (3.1). This solution has a conductivity of 1 000 µS cm-1 at 20 °C. It should not be kept for more than three months.4. PROCEDURE

4.1. Preparation of the sample to be analysed

Use the solution with a total sugar concentration of 25 % (m/m) (25° Brix) as described in the chapter 'pH', section 4.1.2.4.2. Determination of conductivity

Bring the sample to be analysed to 20 °C by immersion in a waterbath. Maintain the temperature to within ± 0,1 °C.

Rinse the conductivity cell twice with the solution to be examined.

Measure the conductivity and express the result in µS cm-1.
5. EXPRESSION OF RESULTS

The result is expressed in microsiemens per cm (µS cm-1) at 20 °C to the nearest whole number for the 25 % (m/m) (25° Brix) solution of rectified concentrated must.5.1.Calculations

If the apparatus is not provided with means for controlling the temperature, correct the measured conductivity using Table I. If the temperature is below 20 °C, add the correction; if the temperature is above 20 °C, subtract the correction.
TABLE I Corrections to be made to the conductivity for temperatures different from 20 °C (µS cm-1)

ConductivityTemperature (°C)20,2
19,820,4
19,620,6
19,420,8
19,221,0
19,021,2
18,821,4
18,621,6
18,421,8
18,222,0 (1)
18,0 (2) 0000 0 0 0 0 0 0 0 50001 1 1 1 1 2 2 2100011 2 2 3 3 3 4 4150112 3 3 4 5 5 6 7200123 3 4 5 6 7 8 9250123 4 6 7 8 91011300134 5 7 8 9111213350135 6 8 911121415400235 7 91112141618450236 8101214161820500247
911131518202255025710121417192224600358111316182124 26(1) Subtract the correction.
(2) Add the correction.
(c) HYDROXYMETHYLFURFURAL (HMF)

1. PRINCIPLE OF THE METHODS

1.1. Colorimetric method

Aldehydes derived from furan, the main one being hydroxymethylfurfural, react with barbituric acid and paratoluidine to give a red compound which is determined by colorimetry at 550 nm.1.2. High-performance liquid chromatography (HPLC)

Separation through a column by reversed-phase chromatography and determination at 280 nm.2. COLORIMETRIC METHOD

2.1. Apparatus

2.1.1. Spectrophotometer for making measurements between 300 and 700 nm.

2.1.2. Glass cells with optical paths of 1 cm.
2.2. Reagents

2.2.1. Barbituric acid, 0,5 % solution (m/v).

Dissolve 500 mg of barbituric acid, C4O3N2H4, in distilled water and heat slightly over a waterbath at 100 °C. Make up to 100 ml with distilled water. The solution keeps for about a week.2.2.2. Paratoluidine solution, 10 % (m/v).

Place 10 g of paratoluidine, C6H4(CH3)NH2, in a 100 ml volumetric flask; add 50 ml of isopropanol, CH3CH(OH)CH3, and 10 ml of glacial acetic acid, CH3COOH (r20 = 1,05 g/ml). Make up to 100 ml with isopropanol. This solution should be renewed daily.2.2.3. Ethanal (acetaldehyde), CH3CHO, 1 % (m/v) aqueous solution.

Prepare just before use.2.2.4. Hydroxymethylfurfural, C6O3H6, 1 g/l aqueous solution.

Prepare successive dilutions containing 5, 10, 20, 30 and 40 mg/l. The 1 g/l and the diluted solutions must be freshly prepared.2.3. Procedure

2.3.1. Preparation of sample

Use the solution obtained by diluting the rectified concentrated must to 40 % (m/v) as described in the chapter 'Total acidity', section 5.1.2. Carry out the determination on 2 ml of this solution.2.3.2. Colorimetric determination

Into each of two 25 ml flasks a and b fitted with ground glass stoppers place 2 ml of the sample prepared as in 2.3.1. Place in each flask 5 ml of paratoluidine solution (2.2.2); mix. Add 1 ml of distilled water to flask b (control) and 1 ml barbituric acid (2.2.1) to flask a. Shake to homogenize. Transfer the contents of the flasks into spectrophotometer cells with optical paths of 1 cm. Zero the absorbence scale using the contents of flask b for a wavelength of 550 nm. Follow the variation in the absorbence of the contents of flask a; record the maximum value A,which is reached after two to five minutes.

Samples with hydroxymethylfurfural concentrations above 30 mg/l must be diluted before the analysis.2.3.3. Preparation of the calibration curve

Place 2 ml of each of the hydroxymethylfurfural solutions with 5, 10, 20, 30 and 40 mg/l (2.2.4) into two sets of 25 ml flasks a and b and treat them as described in 2.3.2.

The graph representing the variation of absorbence with the hydroxymethylfurfural concentration in mg/l is a straight line passing through the origin.2.4. Expression of results

The hydroxymethylfurfural concentration in rectified concentrated musts is expressed in milligrams per kilogram of total sugars.
2.4.1. Method of calculation

The hydroxymethylfurfural concentration C mg/l in the sample to be analysed is that concentration on the calibration curve corresponding to the absorbence A measured on the sample.

The hydroxymethylfurfural concentration in milligrams per kilogram of total sugars is given by:whereP = percentage (m/m) concentration of total sugars in the rectified concentrated must.
3. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

3.1. Apparatus

3.1.1. High-performance liquid chromatograph equipped with:

- a loop injector, 5 or 10 µl,- spectrophotometer detector for making measurements at 280 nm,- column of octadecyl-bonded silica (e.g. Bondapak C18 - Corasil, Waters Ass.),- a recorder, possibly an integrator.Flow rate of mobile phase: 1,5 ml/minute.3.1.2. Membrane filtration apparatus, pore diameter 0,45 µm.

3.2. Reagents

3.2.1. Doubly distilled water.

3.2.2. Methanol, CH3OH, distilled or HPLC quality.

3.2.3. Acetic acid CH3COOH, (r = 1,05 g/ml).

3.2.4.Mobile phase: water-methanol (3.2.2)-acetic acid (3.2.3) previously filtered through a membrane filter (0,45 µm), (40:9:1 v/v).

This mobile phase must be prepared daily and outgassed befor use.

3.2.5. Reference solution of hydroxymethylfurfural, 25 mg/l (v/v).

Into a 100-ml volumetric flask, place 25 mg of hydroxymethylfurfural, C6H3O6, accurately weighed, and make up to the mark with methanol (3.2.2). Dilute this solution 1:10 with methanol (3.2.2) and filter through a membrane filter (0,45 µm).

If kept in a brown glass bottle in a refrigerator, this solution will keep for two to three months.3.3. Procedure

3.3.1. Preparation of sample

Use the solution obtained by diluting the rectified concentrated must to 40 % (m/v) as described in the chapter 'Total acidity', section 5.1.2, and filter it through a 0,45 µm membrane filter.
3.3.2. Chromatographic determination

Inject 5 (or 10) µl of the sample prepared as described in 3.3.1. and 5 (or 10) µl of the reference hydroxymethylfurfural solution (3.2.5) into the chromatograph. Record the chromatogram.

The retention time of hydroxymethylfurfural is approximately six to seven minutes.3.4. EXPRESSION OF RESULTS

The hydroxymethylfurfural concentration in rectified concentrated musts is expressed in milligrams per kilogram of total sugars.3.4.1. Method of calculation

Let the hydroxymethylfurfural concentration in the 40 % (m/v) solution of the rectified concentrated must be C mg/l.

The hydroxymethylfurfural concentration in milligrams per kilogram of total sugars is given by:where P = percentage (m/m) concentration of total sugars in the rectified concentrated must.(d) HEAVY METALS

1. PRINCIPLE OF THE METHODS

I. Rapid method for evaluation of heavy metals

Heavy metals are revealed in the suitably diluted rectified concentrated must by the coloration produced by the formation of sulphides. They are assessed by comparison with a standard lead solution corresponding to the maximum admissible concentration.II. Determination of lead content by atomic absorption spectrophotometry

The chelate given by lead with ammonium pyrrolidinedithiocarbamate is extracted with methylisobutylketone and the absorbence measured at 283,3 nm. The lead content is determined by using known additional amounts of lead in a set of reference solutions.2. RAPID METHOD FOR EVALUATION OF HEAVY METALS

2.1. Reagents

2.1.1. Dilute hydrochloric acid, 70 % (m/v).

Take 70 g of hydrochloric acid, HCl (r20 = 1,16 to 1,19 g/ml), and make up to 100 ml with water.2.1.2. Dilute hydrochloric acid, 20 % (m/v).

Take 20 g of hydrochloric acid, HCl (r20 = 1,16 to 1,19 g/ml), and make up to 100 ml with water.2.1.3. Dilute ammonia. Take 14 g of ammonia, NH3 (r20 = 0,931 to 0,934 g/ml) and make up to 100 ml with water.
2.1.4. pH 3,5 buffer solution.

Dissolve 25 g of ammonium acetate CH3COONH4, in 25 ml of water and add 38 ml of dilute hydrochloric acid (2.1.1). Adjust the pH if necessary with the dilute hydrochloric acid (2.1.2) or the dilute ammonia (2.1.3) and make up to 100 ml with water.2.1.5. Thioacetamide solution C2H5 SN, 4 % (m/v).

2.1.6. Glycerol solution, C3H8O3, 85 % (m/v), (n20 °CD = 1,449 to 1,455).2.1.7. Thioacetamide reagent.

To 0,2 ml of thioacetamide solution (2.1.5) add 1 ml of a mixture of 5 ml of water, 15 ml of 1 M sodium hydroxide solution and 20 ml of glycerol (2.1.6). Heat on a waterbath at 100 °C for 20 seconds. Prepare just before use.2.1.8. Solution containing 0,002 g/l of lead.

Prepare a 1 g/l lead solution by dissolving 0,400 g of lead nitrate, Pb(NO3)2, in water and making up to 250 ml with water. At the time of use, dilute this solution with water to two parts in 1 000 (v/v) in order to obtain a 0,002 g/l solution.2.2. Procedure

Dissolve a test sample of 10 g of the rectified concentrated must in 10 ml of water. Add 2 ml of the pH 3,5 buffer solution (2.1.4); mix. Add 1,2 ml of the thioacetamide reagent (2.1.7). Mix at once. Prepare the control under the same conditions by using 10 ml of the 0,002 g/l lead solution (2.1.8).

After two minutes, any brown coloration of the rectified concentrated must solution should not be more intense than that of the control. 2.3. Calculations

Under the conditions of the above procedure, the control sample corresponds to a maximum admissible heavy metal concentration expressed as lead of 2 mg/kg of rectified concentrated must.3. DETERMINATION OF LEAD CONTENT BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

3.1. Apparatus

3.1.1. Atomic absorption spectrophotometer equipped with an air-acetylene burner.

3.1.2. Lead hollow cathode lamp.3.2. Reagents

3.2.1. Dilute acetic acid.

Take 12 g of glacial acetic acid (r = 1,05 g/ml) and make up to 100 ml with water.

3.2.2. Solution of ammonium pyrrolidinedithiocarbamate, C5H12N2S2, 1 % (m/v).

3.2.3. Methylisobutylketone, (CH3)2CHCH2COCH3.

3.2.4. Solution containing 0,010 g/l of lead.

Dilute the 1 g/l lead solution (of 2.1.8) to 1 % (v/v).3.3. Procedure

3.3.1.Preparation of solution to be examined

Dissolve 10 g of rectified concentrated must in a mixture of equal volumes of dilute acetic acid (3.2.1) and water, and make up to 100 ml with this mixture.

Add 2 ml of ammonium pyrrolidinedithiocarbamate solution (3.2.2) and 10 ml of methylisobutylketone (3.2.3). Shake for 30 seconds while protected from bright light. Leave the two layers to separate. Use the methylisobutylketone layer.3.3.2. Preparation of reference solutions

Prepare three reference solutions containing, in addition to 10 g of rectified concentrated must, 1, 2 and 3 ml respectively of the solution containing 0,010 g/l of lead (3.2.4). Treat these in the same way as the solution to be examined.3.3.3. Control

Prepare a control by proceeding under the same conditions as in 3.3.1, but without the addition of the rectified concentrated must.3.3.4. Determination

Set the wavelength to 283,3 nm.

Atomize the methylisobutylketone from the control sample in the flame and zero the absorbence scale.

By operating with their respective solvent extracts, determine the absorbences of the solution to be examined and the reference solutions.3.4. Expression of results

Express the lead content in milligrams per kilogram of rectified concentrated must to one decimal place.3.4.1. Calculations

Plot the curve giving the variation in absorbence as a function of the lead concentration added to the reference solutions, zero concentration corresponding to the solution to be examined.

Extrapolate the straight line joining the points until it cuts the negative part of the concentration axis. The distance of the point of intersection from the origin gives the lead concentration in the solution to be examined.(e) CHEMICAL DETERMINATION OF ETHANOL

This method is used for the determination of the alcoholic strength of low-alcohol liquids such as musts, concentrated musts and rectified concentrated musts.
1. PRINCIPLE OF THE METHOD

Simple distillation of the liquid. Oxidation of the ethanol in the distillate by potassium dichromate. Titration of the excess dichromate with an iron (II) solution.2. APPARATUS

2.1. Use the distillation apparatus described in the chapter 'Alcoholic strength by volume', section 3.2.3. REAGENTS

3.1. Potassium dichromate solution.

Dissolve 33,600 g of potassium dichromate, $K_2Cr_2O_7$, in sufficient quantity of water to make one litre of solution at 20 °C.

One millilitre of this solution oxidizes 7,8924 mg of alcohol.3.2. Iron (II) ammonium sulphate solution.

Dissolve 135 g of iron (II) ammonium sulphate, $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6\ H_2O$, in sufficient quantity of water to make one litre of solution and add 20 ml of concentrated sulphuric acid, H_2SO_4 (r_{20} = 1,84 g/ml). This solution more or less corresponds to half its volume of dichromate solution when just prepared. Subsequently, it oxidizes slowly.3.3.Potassium permanganate solution.

Dissolve 1,088 g of potassium permanganate, $KMnO_4$, in a sufficient quantity of water to make one litre of solution.3.4. Dilute sulphuric acid, 1:2 (v/v).

A little at a time and stirring continuously, add 500 ml of sulphuric acid, H_2SO_4 (r_{20} = 1,84 g/ml) to 500 ml of water.3.5. Ferrous orthophenanthroline reagent.

Dissolve 0,695 g of ferrous sulphate, $FeSO_4 \cdot 7\ H_2O$, in 100 ml of water, and add 1,485 g of orthophenanthroline monohydrate, $C_{12}H_8N_2 \cdot H_2O$. Heat to help the dissolution. This bright red solution keeps well.4. PROCEDURE

4.1.Distillation

Place 100 g of rectified concentrated must and 100 ml of water in the distillation flask. Collect the distillate in a 100 ml volumetric flask and make up to the mark with water.4.2. Oxidation

Take a flask with a ground glass stopper and with a widened neck enabling the neck to be rinsed without loss. In the flask, place 20 ml of the titrant potassium dichromate solution (3.1) and 20 ml of the 1:2 (v/v) dilute sulphuric acid (3.4) and shake. Add 20 ml of the distillate. Stopper the flask, shake, and wait at least 30 minutes, shaking occasionally. (This is the 'measurement' flask.)

Carry out the titration of the iron (II) ammonium sulphate solution (3.2) with respect to the potassium dichromate solution by placing in an identical flask the same quantities of reagents but replacing the 20 ml of distillate by 20 ml of distilled water. (This is the 'control' flask.)4.3. Titration

Add four drops of the orthophenanthroline reagent (3.5) to the contents of the 'measurement' flask. Titrate the excess dichromate by adding to it the iron (II) ammonium sulphate solution (3.2). Stop adding the ferrous solution when the mixture changes from green-blue to brown.

To judge the end-point more precisely, change the colour of the mixture back from brown to green-blue with the potassium permanganate solution (3.3). Subtract a tenth of the volume of this solution used from the volume of the iron (II) solution added. Let the difference be n ml.

Proceed in the same way with the 'control' flask. Let n2 ml be the difference here.5. EXPRESSION OF RESULTS

The ethanol is expressed in grams per kilogram of sugar and is quoted to one decimal place.5.1. Method of calculation

n2ml of ferrous solution reduces 20 ml of dichromate solution which oxidizes 157,85 mg of pure ethanol.

One millilitre of iron (II) solution has the same reducing power as n n2 ml of iron (II) solution have the same reducing power asEthanol concentration in g/kg of rectified concentrated must is given by:Ethanol concentration in g/kg of total sugars is given by:where P = percentage concentration (m/m) of total sugars.(f) MESO-INOSITOL, SCYLLO-INOSITOL AND SUCROSE

1. PRINCIPLE

Gas chromatography of silylated derivatives.
2. REAGENTS

2.1. Internal standard: xylitol (aqueous solution of about 10 g/l to which a spatula tip of sodium azide is added)

2.2. Bis(trimethylsilyl)trifluoroacetamide - BSTFA - ($C_8H_{18}F_3NOSi_2$)

2.3. Trimethylchlorosilane (C_3H_9ClSi)

2.4. Pyridine p.A. (C_5H_5N)

2.5 Meso-inositol ($C_6H_{12}O_6$)3. APPARATUS

3.1. Gas chromatograph equipped with:

3.2. Capillary column (e.g. in fused silica, coated with OV 1, film thickness of 0,15 µm, length 25 m and internal diameter of 0,3 mm)

Operating conditions:- carrier gas: hydrogen or helium,- carrier gas flow rate: about 2 ml/minute,- injector and detector temperature: 300 °C,- programming of temperature: 1 minute at 160 °C, 4 °C per minute to 260 °C, constant temperature of 260 °C for 15 minutes,- splitter ratio: about 1:20.

3.3. Integrator.

3.4. Microsyringe, 10 µl.

3.5. Micropipettes, 50, 100 and 200 µl.

3.6. 2 ml flask with Teflon stopper.

3.7. Oven.4. METHOD OF OPERATION

An accurately weighed sample of about 5 g of rectified concentrated must is placed in a 50 ml flask. 1 µl of standard solution of xylitol (2.1) is added and water added to capacity. After mixing, 100 µl of solution is taken and placed in a flask (3.6) where it is dried under a gentle stream of air. 100 µl of absolute ethyl alcohol may be added if necessary to facilitate evaporation.

The residue is carefully dissolved in 100 µl of pyridine (2.4) and 100 µl of bis(trimethylsilyl)trifluoroacetamide (2.2) and 10 µl of trimethylchlorosilane (2.3) are added. The flask is closed with the Teflon stopper and heated at 60 °C for one hour.

Draw off 0,5 µl of clear fluid and inject using a heated hollow needle in accordance with the stated splitter ratio.5. CALCULATION OF RESULTS

5.1. A solution is prepared containing:60 g/l of glucose, 60 g/l of fructose, 1 g/l of meso-inositol and 1 g/l of sucrose.5 g of the solution is weighed and the procedure at 4 followed. The results for meso-inositol and sucrose with respect to xylitol are calculated from the chromatogram.
In the case of scyllo-inositol, which is not commercially available and has a retention time lying between the last peak of the anomeric form of glucose and the peak for meso-inositol (see diagram overleaf), the same result as for meso-inositol is taken.6. EXPRESSION OF RESULTS

6.1. Meso-inositol and scyllo-inositol are expressed in milligrams per kilogram of sugar. Sucrose is expressed in grams per kilogram of must.

Chromatogram in gaseous phase of meso-inositol, scyllo-inositol and sucrose

(1)Any pycnometer with equivalent characteristics may be used. (2)A numeric example is given in section 6 of this chapter. (3)A numeric example is given in section 6 of this chapter. (4)The sugar concentration is expressed in terms of invert sugar. (5)The sugar concentration is expressed in terms of invert sugar. (6)For example, for an alcoholic strength of 12 % by weight, p= 0,12. (7)Before carrying out this calculation, the specific gravity (or the density) of the wine measured as specified above should be corrected for the effect of the volatile acidity using the formula:

dv = d20 °C
20 °C 0,0000086a orrv = r20 0,0000086a
where a is the volatile acidity expressed in milliequivalents per litre. (8)These values are given pending the creation of a Community data bank of such values. (9)These values are given pending the creation of a Community data bank of such values. (10)One of the trade names is 'Norite'. (11)105 pascal (Pa) = 1 bar. (12)1 Pa = 1 N/m² = 10 5 bar. (13)No account is taken of other gases present (O2, N2, etc.) in amounts that are too small to have any effect on the excess pressure. (14)Certain wines, such as liqueur wines, etc., give a distillate which is not clear even after filtration; in such cases the distillate must be placed in a 200-ml distillation flask, made up to 30 ml with distilled water and distilled while still alkaline, discarding the first 15 ml of the distillate. Cool the contents of the flask, acidify with approximately 5 ml of dilute sulphuric acid and resume the distillation, collecting the distillate in 5 ml of a 1 M sodium hydroxide solution. Distil approximately 5 ml of the liquid, which will then be clear. (15)This addition is optional. Some analysts consider it easier to observe the appearance of turbidity in a pink solution than in a colourless one. (16)n2 is equal to 0,05 or 0,1 ml if the volume of water used is less than 10 ml. To obtain a discernible end point, the volume used should be as small as possible and hence any dilutions should as far as possible be avoided during the main operation. (17)This distance must be given in a direction going from O to C.